25th International Symposium on Regulatory Peptides

Proceedings

American University, Washington D.C.

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CENTER FOR NEUROSCIENCE AND BEHAVIOR
AMERICAN UNIVERSITY • WASHINGTON, DC

RegPep25

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RegPep25 Organizers

Colin Saldanha
Director, Center of Neuroscience and Behavior (CNB) American University, Washington D. C., USA
Limei Zhang, IRPS President
Lee Eiden, IRPS Treasurer
Vito Hernández, IRPS Secretary
Terry Davidson
Past-Director of CNB and RegPep25 Honorary Co-Chair

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Friend of IRPS ($250 and above)
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List of Early Career Awardees *

1. Maleeha Akram, NINDS, NIH, USA
2. Karen L. Claro-Mendoza, Instituto de Biotecnologia, UNAM, México
3. Johanna De la Cruz, NeuroTechnology, Columbia University, USA (Argentina)
4. Angélica Gutierrez-Mata: Instituto de Biotecnologia, UNAM, México
5. Alan Kania, University of Heidelberg, Mannheim, Germany (Poland)
6. David Keller, University of Cologne, Cologne, Germany (Hungary)
7. Elena Kozlova, UC Riverside, CA, USA
8. Adrián Limón, Universidad de Aguascalientes, México
9. Donald MacDonald, NCCIH, NIH, USA
10. Naira Mansano, NINDS, NIH, USA
11. Rebeca Mendez, Monell Chemical Senses Center, Philadelphia, USA (México)
12. Aleksandra Trenk, Jagiellonian University, Krakow, Poland.

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*ECR Award: the RegPep25 POC thanks IBRO, JNE, Arbor Assay and *Sponsors*, *Supporters* and *Friends* of IRPS for their contributions to this fund.

Greetings and the warmest of welcomes to American University

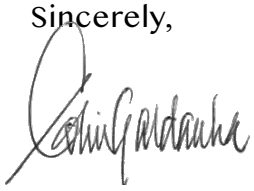
Greetings and the warmest of welcomes to American University. Your presence here is a testament to the resilience and courage of scientists and scholars across the world. For that resilience and courage, I thank you.

You are on a transformed campus. East Campus became operational less than ten years ago, and houses the departments of Mathematics, Physics, Computer and Data Science. To the west, the Hall of Science was completed just five years ago and houses Neuroscience, Chemistry, Biology and Environmental Science. Across American University, the last fifteen years have witnessed a blossoming of the natural sciences and their cross-disciplinary influence.

The Center for Neuroscience and Behavior, is one example of this collaborative engagement. The Center was founded in 2012 under the watchful eyes of Dr. Terry Davidson, and exemplifies the synergism across departments and schools such as Neuroscience, Psychology, the Washington College of Law, and the Schools of Health Studies, Public Affairs, and Business. We are an externally funded, self-sustaining institution under the office of the Vice Provost for Research and Innovation, Dr. Diana Burley. We support and train undergraduate and graduate students in Neuroscience, Psychology and the PhD program in Behavior, Cognition, and Neuroscience.

As I look through the list of attendees at RegPep25, I see many familiar names including colleagues, mentors, collaborators, friends, possible reviewers, and a fellow aging rugby player or two. I am happy to see you all, and I look forward to an engaging, productive, and enjoyable meeting.

Sincerely,



Colin J. Saldanha PhD.
Director – Center for Neuroscience and Behavior
Professor of Neuroscience and Psychology

Welcome Note

It is a profound pleasure to welcome all delegates to American University in Washington D.C. for RegPep25. We are meeting at a fraught time for the global collective scientific enterprise, but with a determination that science will go forward as one of the worthiest and most productive enterprises of which we are collectively capable.

We arrive here to share scientific knowledge, forge collaborative projects, and to be encouraged in our own work and inspired by the achievement of colleagues. It is worth remembering that historically, even in times of great global unrest, scientific conferences have been critical in achieving these critical goals. It is also noteworthy that it has been 50 years since Viktor Mutt and colleagues held the first International Symposium on Gastrointestinal Hormones. In the years since, the focus of what eventually became the International Regulatory Peptide Society, and the biennial International Symposium on Regulatory Peptides has moved on from 'gut hormones' to embrace of the concept of a cadre of remarkable peptides, secreted from multiple endocrine, neuroendocrine and neuronal sites (and even from some only 'honorary' endocrine cells like astrocytes and immunocytes) to regulate systems physiology write large. Fifty years later, we have landed on a detailed and nuanced understanding of how peptides can act as hormones, neuromodulators and neurotransmitters, such that a single peptide molecule, such as GLP-1, can act both in the gut and in the brain, and can regulate not only appetite for food, but appetitive behavior more generally. We have developed increasingly precise tools for manipulating and observing peptide action not only in ex vivo and in vitro systems, but in living organisms and not just in in vitro or ex vivo systems, albeit the former remain fundamental for testing reductionist hypotheses about how peptide participate in regulatory physiology.

RegPep25 will celebrate not only where we are as regulatory peptide researchers, but how we got here, and what the future is likely to hold. We welcome you all to Washington DC along with our co-hosts at American University's Center for Behavioral Neuroscience, and wish you a memorable and intellectually fruitful time here.

Lee. E. Eiden

On behalf of the organizers of RegPep25

Preface

In June 2025, the International Regulatory Peptide Society (IRPS) convened its 25th international meeting—**RegPep25**—at American University in Washington, D.C. This event marked not only the continuation of a longstanding scientific tradition but also the 50th anniversary of a conference series that has shaped the field of peptide biology across generations.

The inaugural meeting, titled "**A Symposium on Gastrointestinal Hormones**," was held on October 9–12, 1974, at the University of Texas Medical Branch in Galveston, Texas (*). That first symposium brought together 41 scientists from 10 countries—a remarkable international gathering for its time. Among the participants were pioneering figures such as Morton I. Grossman, Viktor Mutt, Andrew V. Schally, Basil I. Hirschowitz, Stephen Bloom, and Jens Rehfeld, who played central roles in identifying, characterizing, and contextualizing the first wave of gastrointestinal and neuroendocrine peptides. The photograph below captures many of the founders and early contributors during that foundational event (*).

1st row: Makhlouf, Tract, Larsson, Grossman, Gregory, Thompson, Bodansvky, Chey, McGuigan, Cohen, Andersson. **2nd row:** Gardner, Solcia, Rayford, Adelson, Kelly, Lambert, Johnson, Go, Hansky, Erspamer, Boden. **3rd row:** Said, Debas, Unger, Creutzfeldt, Konturek, Barrowman, Jacobson, Becker, Schofield, Dockray, Straus, Meyer, Galardy, Brown, Olbe, Bloom, Rehfeld.

This tradition of biennial gatherings grew in scale and scope. In 1996, during the 11th meeting in Copenhagen, Denmark, the series formally adopted the name **"International Symposium on Regulatory Peptides" (RegPep)**, reflecting the field's expansion from gastrointestinal hormones to a broader universe of neuropeptides, endocrine peptides, and peptide-based signaling systems in physiology and disease. Over the decades, **RegPep** has evolved into a multigenerational, interdisciplinary, and globally inclusive scientific community. At RegPep25, we proudly celebrated this legacy with over 100 participants from every continent and career stage—from early-career researchers to emeritus pioneers.

This volume collects the abstracts and programmatic content of RegPep25, showcasing the vitality of our field. Topics ranged from peptide-based synaptic organization and intracellular trafficking to neurodevelopment, emotion, metabolism, and neurodegeneration. RegPep25 also highlighted the growing translational relevance of peptide science, including novel therapeutic strategies targeting peptidergic systems.

A personal reflection:

My (Limei's) involvement with RegPep began during my Fulbright visiting tenure at NIMH as a guest researcher in Dr. Lee Eiden's Section on Molecular Neuroscience, 2016. Dr. Eiden spearheaded that year the organization of **RegPep22** in Washington, D.C., but unforeseen challenges arose—notably, the IRPS lacked legal incorporation, complicating fundraising, registration, and venue logistics. With gratitude, we turned to Prof. Germán Fajardo-Dolci, Dean of the School of Medicine at the National University of Mexico (UNAM), who graciously agreed to serve as RegPep22's official host. The administration of FM-UNAM (led by Mr. Luis Arturo González-Nava) and the Mexican Science Foundation (CONACYT, led by Dr. Julia Tagüeña) and our dear friends/colleagues Professors Patricia Joseph-Bravo and Jean-Louis Charli provided invaluable support—**to these colleagues and friends, we remain deeply indebted**.

Since then, we, together with Dr. Lee Eiden, have worked shoulder-to-shoulder for eight years, overcoming innumerable challenges to successfully incorporate the IRPS first in Mexico City and then in Baltimore, Maryland, and organize four RegPep meetings—in Acapulco Diamante, Mexico (RegPep22, RegPep23); in Stirling, Scotland, UK (RegPep24); and now Washington, D.C.—**closing a defining chapter of our professional lives**. We are profoundly moved by our community's warm encouragement, steadfast support, and above all, their trust in us.

We extend heartfelt thanks to the individuals, institutions, and sponsors who made RegPep25 possible—and to the global peptide community, whose dedication has sustained this tradition for half a century. May this volume serve as both a record and an inspiration for future generations.

With gratitude and hope for the future,

Limei Zhang and Vito Hernández
IRPS President and Secretary (2023-2025)
June 23, 2025, Washington D. C.

*Reference: Thompson JC, ed. *Gastrointestinal Hormones: A Symposium*. University of Texas Medical Branch at Galveston; 1975. ISBN: 0292727046.

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Scientific Program

Schedule at a Glance

Monday, June 23, 2025

Breakfast: Terrace Dining Room, Mary Graydon Center (7:00 – 8:00)

Pre-RegPep25 special event
Five Generations of Researchers Celebrating 50 years of RegPep

Constitution Hall, AU

- 8:00 – 09:30 **RegPep young investigator symposium (YIS)**
  Co-Chairs: **Alan Kania** (Germany/Poland) & **Rebeca Mendez** (USA/Mexico)
  YIS1: **Alan Kania** (University of Heidelberg, Germany) (20min) **(*ECR Awardee)**
  "Oxytocin signaling in the nucleus incertus: implications for trans-ventricular brain state modulation"
  YIS2: **Rebeca Mendez** (U. Penn, Philadelphia, USA & Mexico) (20min) **(*ECR Awardee)**
  "Circadian regulation of vagal glucose sensing and insulin secretion by the CART"
  YIS3: **Aleksandra Trenk** (Jagiellonian University, Krakow, Poland) (20min) **(*ECR Awardee)**
  "Divergent neuromodulatory roles of relaxin-3 and oxytocin in the ventral dentate gyrus"
  YIS4: **Donald Macdonald (NIH, USA) (*ECR Awardee)**
  "A genetic strategy to suppress neuropeptide signaling from nociceptors"
  Group discussion (10 min)
- **09:30 - 09:45** **Coffee break**
- 09:45 – 10:30 IRPS DM **Dick Swaab** (Netherlands Institute for Neuroscience, KNAW, Amsterdam, The Netherlands, IRPS DM, introduced by **Ruud Buijs:**
  Neuropeptide pioneers
- 10:30– 11:15 IRPS DM **John Furness** (University of Melbourne, Australia, IRPS DM. introduced by **Dave Grattan**)
  Gut peptides from the beginning and looking to the future
- 11:15 – 11:30 Coffee break
- 11:30 – 12:30 RegPep pioneers roundtable:
  Panelists: Maurice Manning, Bob Millar, Ruud Buijs, Patricia Joseph-Bravo, Dave Grattan, Dayu Lin

Lunch: Terrace Dining Room, Mary Graydon Center (12:30 – 14:00)

RegPep25 Inaugural Ceremony

Constitution Hall, American University

- 14:00 - 14:15 Welcome from the AU-CNB and introduction of RegPep25 by the Co-Chairs
- 14:00 - 15:00 **PL1:** Inaugural Plenary Lecture 1: **Michael Greenberg** (Harvard University, USA)
  2023 Brain Prize Laureate
  Introduced by **Lee Eiden** (RegPep25 Co-Chair)
  Sensory experience-dependent regulation of neuropeptides in learning and memory
- 15:00 - 15:15 Music Interlude 1: **Cellist AJ Umunna**, American University
- 15:15 - 16:00 **PL2:** Inaugural Plenary Lecture 2: **Zhou-Feng Chen** (Shenzhen Bay Laboratory, China)
  Introduced by **Limei Zhang** (IRPS President)
  "Neuropeptide coding of itch, pain and touch"
- 16:00 - 16:15 Music Interlude 2: **Cellist AJ Umunna**, American University
- 16:15 - 16:50 **PL3:** Lay lecture: **Colin Saldanha** (Director, Center for Neuroscience and Behavior, AU, USA)
  Introduced by **Rea Silver** (Columbia University)
  "The challenges of specificity in secreted signaling to integrate physiological processes"

- 17:00 – 17:30 Group photo
- 17:30 – 21:00 Welcome Reception & Poster (Datablitz 19:00)
  Mary Graydon Center, AU

Schedule at a Glance

Tuesday, June 24, 2025

- 7:00 - 8:00 Breakfast, Terrace Dining Room – Mary Graydon Center

Room A (Scientific session SS)

SS1: Relaxin Family Peptides: From Brain Circuits to Therapeutic Frontiers

Chair: **Juan Marugan** (NCATS, NIH, USA)

08:00 - 08:05 Introduction

08:05 - 08:30 **Akhter Hossain** (Melbourne, Australia)
"RXFP4 and INSL5, physiological roles and therapeutic implications"

08:30 - 08:55 **Irina Agoulnik** (Florida, USA)
"INSL3 and its receptor RXFP2"

08:55 - 09:20 **Francisco Olucha-Bordonau** (Castellón, Spain)
"Connectivity of relaxin-3 projections related to cognitive and emotional processes"

09:20 - 09:45 **Ross Bathgate** (Australia)
"Therapeutic targeting of the relaxin receptor, RXFP1"

09:45 - 09:50 General discussion/conclusion

Room B (Scientific session SS)

SS2: Neuropeptidergic Control of Reproduction and Beyond: Insights from Kisspeptin and Oxytocin Circuits

Chair: **Mike Lehman** (USA)

08:00 - 08:05 Introduction

08:05 - 08:30 **Martin Kelly** (Oregon, USA)
"The Role of Hypothalamic Arcuate Kisspeptin Neurons as the Gatekeepers of Fertility"

08:30 - 08:55 **Michael Lehman** (Ohio, USA)
"KNDy neurons of the hypothalamus: an update of their roles in health and disease"

08:55 - 09:20 **Vito Hernandez** (UNAM, Mexico)
"Kisspeptin Signaling Beyond Reproduction: Chemoanatomical Characterization and Functional Insights"

09:20 - 09:45 **Ryoichi Teruyama** (Louisiana State Univ, Baton Rouge, USA)
"Sexually dimorphic oxytocin system in the CNS"

09:45 - 09:50 General discussion/conclusion

Short break

SS3: Neuropeptides and Social Motivation: Circuits Shaping Sex Behavior, and Choice

Chair: **David Keller (Germany)**

10:00 - 10:05 Introduction

10:05 - 10:30 **Aras Petrulis** (GSU, USA)
"Vasopressin in the lateral septum drives sex-specific social interest"

10:30 - 10:55 **Mario Gil** (Texas, USA)
"Regulation of social and sexual behaviors by neurohypophysial hormones in the rodent forebrain and midbrain "

10:55 - 11:20 **David Keller** (University of Cologne, Germany)
"Neuronal populations in the lateral septum regulate sex-dependent social interactions and feeding behavior "
"

11:20 - 11:45 **Ki Goosens** (Icahn School of Medicine at Mount Sinai, USA)
" Regulation of complex decision-making by ghrelin"

11:45 - 11:50 General discussion/conclusion

SS4: Neuropeptides and Emotional Brain States: From Circuit Salience to Synaptic Precision

Chair: **Francesco Ferraguti (Austria/Italy)**

10:00 - 10:05 Introduction

10:05 - 10:30 **Norbert Hajos** (IN, USA)
"VIP-containing midbrain input to central amygdala controls contextual fear memory formation"

10:30 - 10:55 **Claudia Schmuckermair** (U. Innsbruck, Austria)
"Control of salience detection by VIPergic insular interneurons"

10:55 - 11:20 **Limei Zhang** (UNAM, Mexico)
"Neuropeptide in fast and slow synaptic transmission: A discovery of a calyx of Held-like synapse in the rodent forebrain"

11:20 - 11:45 **Anna Blasiak** (Jagiellonian University, Krakow, Poland)
"Interplay of relaxin-3 and oxytocin systems in shaping ventral hippocampus neuronal activity - possible involvement in anxiety control in rat and human"

11:45 - 11:50 General discussion/conclusion

- 12:00 - 13:00 Lunch, Terrace Dining Room – Mary Graydon Center

- 13:00 – 13:45 **PL4: Dayu Lin** (New York University, USA) introduced by **Dave Grattan**
"The neural mechanisms underlying the rise and fall of maternal aggression"

Short break

- 14:00 – 16:00 **KS1:** Keynote symposium 1. Chair: **Limei Zhang** (IRPS President)

Wilson Compton (Deputy director of NIDA, NIH, USA)
"Harnessing GLP-1 agonists to understand and treat addictions"

William Wisden (Imperial College, London, UK)
"The impact of molecular neurobiology on modern neuroendocrinology"

Luis de Lecea (Stanford School of Medicine, USA)
"Neuropeptides and control of behavioral states"

Short break

- 16:10 – 17:30 **Workshop on translational aspects of peptide GPCRs.** Chair: **Bob Millar**

Bob Millar (University of Pretoria, South Africa)
"Rescue of mutant peptide GPCRs in the HPG axis with small molecules: a more viable therapy than gene editing"

Terry Moody (NIH, USA)
"Leveraging receptor visualization to cancer treatment: bombesin and theragnostics"

Special event: IRPS Executive Reception)

Schedule at a Glance

Wednesday, June 25, 2025

- 7:00 - 8:00 Breakfast: Terrace Dining Room – Mary Graydon Center

Room A (Scientific session SS)

SS5: Peptides Involved in Energy Regulation

Chair: **Jean-Louis Charli** (Mexico)

08:00 - 08:05 Introduction

08:05 - 08:30 **Jean-Louis Charli** (UNAM, Mexico)
"The TRH degrading ectopeptidase and energy homeostasis"

08:30 - 08:55 **Patricia Joseph-Bravo** (UNAM, Mexico)
"Sex-dependent regulation of TRH and thyroid axis by food, cold, exercise and stress-related energy demands"

08:55 - 09:20 **Margarita Curras-Collazo** (UC Riverside, USA)
"CCK-AR-expressing Vagal Sensory Afferents of the Gut-Brain Axis Contribute to Pro-inflammatory IL-6 Response to LPS"

09:20 - 09:45 **Lucila K. Elias** (Uni. Sao Paolo, Ribeiro Preto, Brazil)
"Neuroendocrine regulation of energy homeostasis"

09:45 - 09:50 General discussion/conclusion

Room B (Scientific session SS)

SS6: Neuropeptidergic Integration of Synaptic Plasticity Emotion, Fear, and Social Behavior

Chair: **Joanna Dabrowska** (USA)

08:00 - 08:05 Introduction

08:05 - 08:30 **Pablo Castillo** (Albert Einstein College of Medicine, USA)
"Presynaptic BDNF/TrkB Signaling Drives Axonal Local Translation Essential for Long-Term Plasticity"

08:30 - 08:55 **Eric G. Krause** (Georgia State University, USA)
"The integration of interoceptive signals and behavioral responses via oxytocin receptors"

08:55 - 09:20 **Zhihua Gao** (Zhejiang University, China)
"The oxytocinergic and behavioral signatures of lactation"

09:20 - 09:45 **Joanna Dabrowska** (Rosalind Franklin Univ., USA)
"Vasopressin and oxytocin integrate interoceptive signals and fear memory in extended amygdala"

09:45 - 09:50 General discussion/conclusion

Short break: coffee

SS7: Neuropeptides and Hormonal Networks in Systemic Physiological Regulation

Chair: **Teresa Morales** (UNAM, Mexico)

10:00 - 10:05 Introduction

10:05 - 10:30 **Eric Lazartigues** (Louisiana, USA)
"miRNA targeting for the renin-angiotensin system"

10:30 - 10:55 **Paul Marvar** (Washington DC, USA)
"The Brain Angiotensin System: Connecting Cognition, Stress, and Cardiovascular Disease"

10:55 - 11:20 **Lei Xiao** (Fudan University, China)
"Hypothalamic Oxytocinergic System-Neurons, circuits and functions"

11:20 - 11:45 **Teresa Morales** (Mexico)
"Prolactin Dysregulation in a Mice Model of Kidney Disease"

11:45 - 11:50 General discussion/conclusion

SS8: Neuropeptides and the Architecture of Sleep: Circuits, Clocks, and Cognitive Health

Chair: **Bill Wisden** (Imperial College, London, UK)

10:00 - 10:05 Introduction

10:05 - 10:30 **Ruud Buijs** (UNAM, Mexico)
"Vasopressin as neurotransmitter of the biological clock signals the rest period in physiology and activity"

10:30 - 10:55 **Kyoko Tossell** (Imperial College, London, UK)
"Role of prefrontal cortex somatostatin neurons directing top-down control of sleep preparatory behavior and sleep"

10:55 - 11:20 **Jason Rihel** (UCL, UK)
"Zebrafish as a model system to study role of galanin in regulating sleep homeostasis".

11:20 - 11:45 **Sara Calafate** (Portugal)
"Sleep-dependent modlation of brain homeostasis in Alzheimer's Disease"

11:45 - 11:50 General discussion/conclusion

- 12:00 - 13:00 Lunch, Terrace Dining Room (TDR), Mary Graydon Center
- 13:00 – 13:45 **PL5: Scott Kanoski** (USC, USA Viktor Mutt Lecturer) Introduced by Terry Davidson
  Presenter: Terry Davison (USA)
  "Peptide control of energy balance"

Short break

- 14:00 – 16:40 **KS2:** Keynote symposium 1 Chair: **Colin Saldanha** (Director, CNB, AU)

  Rae Silver (Columbia University, USA)
  Circadian rhythms and the portal pathways of circumventricular organs"

  Susan Wray (NIH, USA)
  LHRH Secretion: Why so many modulators?"

  David Grattan (Otago University, New Zealand)
  What Prolactin teaches us about the evolution of peptide physiology

  Sung Han (Salk Institute, USA)
  Frequency-dependent transmitter switching in a peptidergic circuit

Short break

16:45 – 17:30 Round table: Peer Review & Scientific Publication:
An Open Discussion
(Co-Chaired: Tom Cunningham & Lee Eiden)

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Special event: IRPS Council and Trustees meeting

Schedule at a Glance

Thursday, June 26, 2025

- 7:00 - 8:00 Breakfast (buffet), Terrace Dining Room

Room A (Scientific session SS)

SS9: Control of peptides regulating appetitive drives and energy homeostasis

Chair: **Andras H. Leko** (Hungary)

08:00 - 08:05 Introduction

08:05 - 08:30 **Denise Belsham** (UT, Canada)
"Neuropeptide regulation by hypothalamic microRNAs"

08:30 - 08:55 **Andrew Lutas** (NIDDK, NIH, USA)
"cAMP-dependent mechanisms in GLP1R-expressing hindbrain neurons that cause weight loss"

08:55 - 09:20 **Andras Leko** (Semmelweis Univ., Hungary)
"Sexually dimorphic effects of GHSR in diet-induced obesity"

09:20 - 09:45 **John Furness** (Florey Institute, Australia)
"Roles of the ghrelin receptor: investigation of its mechanism in reversing agonist action at the D2 dopamine receptor"

09:45 - 09:50 General discussion/conclusion

Room A (Scientific session SS)

SS10: PACAP signaling across systems: from stress circuits to metabolic control

Co-Chair: **Jessica Barsson & Lee Eiden** (USA)

08:00 - 08:05 Introduction

08:05 - 08:30 **Arun Anantharam** (University of Toledo, USA)
"Mechanisms for PACAP-induced depolarization leading to chromaffin cell secretion"

08:30 - 08:55 **Youssef Annuar** (Univ Rouen Normandie, Inserm, U1239, France)
Central SELENOT deficiency alters GnRH levels, sexual behavior and fertility in male and female mice

08:55 - 09:20 **Jessica Barson** (Drexel University, USA)
"PACAP in the paraventricular nucleus of the thalamus: Relationship with ethanol drinking and dependence".

09:20 - 09:45 **Sunny Jiang** (NIMH, USA)
"Novel prefrontal cortico-hypothalamic PACAPergic projection modulates CRH neurons in PVN"

09:45 - 09:50 General discussion/conclusion

Short break

SS11: PTH2 and Catestatin: Novel peptidergic modulators of brain and behavior

Chairs: **Arpad Dobolyi** (Hungary)

10:00 - 10:05 Introduction

10:05 - 10:30 **Gina Puska** (Univ. Veterinary Medicine, Hungary)
"PTH2 (TIP39) plays a role in the control of maternal behavior"

10:30 – 10:55 **Lukas Anneser** (Friedrich Miescher Inst. for Biomed. Research., Switzerland)
"There are other fish in the sea: Social density encoding by the neuropeptide PTH2"

10:55 - 11:20 **Arpad Dobolyi** (Eotvos Lorand Univ., Hungary)
"Single nucleus sequencing of the human arcuate nucleus: insights into the role of PTH2 in prolactin secretion"

11:20 - 11:45 **Sushil Mahata** (UCSD, USA)
"Chromogranin A deficiency and catestatin supplementation improve tauopathy and cognitive functions in PS19 Mice"

11::45 - 12:00 General discussion/conclusion

SS12: Neuropeptides in mood disorders: mechanisms linking emotion, sex, and brain state

Chair: **Tom Cunningham** (USA)
10:00 - 10:05 Introduction
10:05 - 10:30 **Tom Cunningham** (Texas A&M, USA)
"Sex Differences in Vasopressin in an animal model of hyponatremia"

10:30 - 10:55 **Mario Zetter** (UNAM/LaSalle University, Mexico) (20min) (*ECR Awardee)
Adult neurogenesis and migration in magnocellular AVP system"

10:55 - 11:20 **Hiroe Hu** (NIH, USA)
"Re-examining vasopressin's role in HPA-axis stress response in the context of depression and suicidality

11:20 - 11:45 **Aimin Bao** (Zhejian University, China)
"The role of oxytocin in bipolar disorder: from animal model to postmortem human brain"
* Jing Lu (Zhejiang University, China) recording : Hypocretin-1/hypocretin receptor 1 regulates neuroplasticity and cognitive function through hippocampal lactate homeostasis in depressed model
11:45 - 12:00 General discussion/conclusion

- 12:00 - 13:00 Lunch (buffet), Terrace Dining Room

- 13:00 - 13:45 **PL6: Vincent Prevot** (INSERM, France) Introduced by **Ruud Buijs**
"A role for GnRH in the control of cognition… and more?"

Visit to the **Smithsonian National Air and Space Museum's Steven F. Udvar-Hazy Center,** Chantilly, Virginia

Gala dinner (18:30-21:00)

Schedule at a Glance

Friday, June 27, 2025

- 7:00 - 8:00 Breakfast (buffet), Terrace Dining Room

- **08:00-8:45 SL1 Neuroscience Special Lecture sponsored by JNE/BSN**

Xiao-Ke Chen (Stanford University, USA)

Introduced by **Lee Eiden** (NIMH, NIH, USA)

"Descending peptidergic control of chronic mechanical pain"

IRPS General Assembly (09:00-10:00)

- **10:00-10:45 SL-2 Neuroscience Special Lecture sponsored by IBRO**

Hugo Tejeda (NIMH-IRP, NIH, USA)

Presenter: **Susan Wray** (NIH, USA)

"Prefrontal cortical neuropeptidergic control of threat processing and circuit function"

Closing Lecture (10:45-11:30)

Dick Swaab
(Netherlands Institute for Neuroscience, KNAW, Amsterdam, The Netherlands)

Presenter: **Ruud Buijs** (UNAM, Mexico)

"Dead brains tell lively stories"

Monday June 23, morning

Pre-RegPep25 special event

Five Generations of Researchers Celebrating 50 years of RegPep

Constitution Hall, AU

8:00 – 09:30 RegPep young investigator symposium (YIS)

Co-Chairs: **Alan Kania** & **Rebeca Mendez**

YIS1: **Alan Kania** (University of Heidelberg, Germany)
Oxytocin signaling in the nucleus incertus: implications for trans-ventricular brain state modulation

YIS2: **Rebeca Mendez** (U. Penn, Philadelphia, USA & Mexico)
Circadian regulation of vagal glucose sensing and insulin secretion by the CART

YIS3: **Aleksandra Trenk** (Jagiellonian University, Krakow, Poland)
Divergent neuromodulatory roles of relaxin-3 and oxytocin in the ventral dentate gyrus

YIS4: **Donald Macdonald** (NIH, USA)
A genetic strategy to suppress neuropeptide signaling from nociceptors

Group discussion

09:30 – 09:45 Coffee break

09:45 – 10:30 **Dick Swaab** (Netherlands Institute for Neuroscience, KNAW, Amsterdam, The Netherlands, (introduced by **Ruud Buijs**)
Neuropeptide pioneers

10:30– 11:15 **John Furness** (University of Melbourne, Australia, introduced by **Dave Grattan**
Gut peptides from the beginning and looking to the future

11:15 – 11:30 Coffee break

11:30 – 12:30 RegPep pioneers roundtable with panelist:
Ruud Buijs
Dave Grattan
Patricia Joseph-Bravo
Dayu Lin
Maurice Manning
Bob Millar

Young Investigator Symposium (YIS)

YIS-1: Oxytocin signaling in the nucleus Incertus: Implications for trans-ventricular brain state modulation

Kania, Alan, 1, 2, 3; Afordakos, Konstantinos, 1; Drwiega, Gniewosz, 2; Pradel, Kamil, 2; Trenk, Aleksandra, 2; Gugula, Anna, 2; Chrobok, Lukasz, 2, 4; Lefevre, Arthur, 1; Krabichler, Quirin, 1; Eliava, Marina, 1; Ma, Sherie, 5; Cifani, Carlo, 3; Gundlach, Andrew L., 5; Blasiak, Anna, 2; Grinevich, Valery, 1

1 Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany; 2 Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; 3 School of Pharmacy, Pharmacology Unit, University of Camerino, Camerino, Italy; 4 School of Physiology, Pharmacology, and Neuroscience, Faculty of Life Sciences, University of Bristol, Bristol, UK; 5 The Florey Institute of Neuroscience and Mental Health, and Florey Department of Neuroscience and Mental Health, The University of Melbourne, Victoria, Australia

Oxytocin (OT) and its receptor (OTR) regulate a wide range of physiological and behavioral processes, including social interactions, reproduction, homeostasis, learning, and emotional regulation. While OT's role in social behaviors is well established, its involvement in brain arousal—an essential state for attention and adaptive behavior—remains poorly understood. The nucleus incertus (NI), a brainstem structure implicated in arousal regulation, also modulates several functions influenced by the OT/OTR system. To investigate the potential contribution of OT/OTR signaling to NI activity and its role in arousal control, we conducted a series of experiments in rats integrating histological, anatomical, electrophysiological, imaging, and behavioral approaches.

RNAscope in situ hybridization revealed that 88% of OTR-expressing NI neurons are GABAergic. Furthermore, all relaxin-3 (RLN3) and cholecystokinin (CCK) neurons in the NI co-express OTR mRNA. Electrophysiological recordings demonstrated a dose-dependent excitatory response to OT in 70% of NI neurons, which was abolished by OTR antagonists, confirming receptor-specific activation. Chemogenetic activation of NI OTR neurons significantly increased locomotor activity in rats, highlighting their role in behavioral activation. To explore the anatomical pathways of OT delivery to the NI, we employed viral tracing and immunohistochemistry. These techniques revealed no direct OTergic innervation of the NI and an absence of OT-immunoreactive fibers, supporting the hypothesis of cerebrospinal fluid (CSF)-mediated diffusion. Intracerebroventricular (ICV) OT administration induced significant pupil dilation—a classic marker of arousal—further implicating OT in arousal modulation. Additionally, OT sensor imaging confirmed the diffusion of OT to the NI following ICV infusion.

Our findings establish NI OTR neurons as key regulators of arousal and support the role of CSF-mediated volume transmission in OT/OTR signaling within this brainstem region. These insights expand our understanding of neuropeptide pathways in the brain and their influence on behavior.

AK was supported by Humboldt Research Fellowship, Alexander von Humboldt Foundation, Germany, National Science Centre, Poland UMO-2023/49/B/NZ4/01885 to AB, Synergy European Research Council (ERC) grant "OxytocINspace" 101071777, German-Israeli Project cooperation (DIP) GR3619/16-1, and ERANET-Neuron GR3619/25-1 to VG

YIS-2: Circadian regulation of vagal glucose sensing and insulin secretion by the neuropeptide CART

Mendez-Hernandez, R., 1, 2; Woodie, L., 3, 4; Lazar, M., 3; de Lartigue, G., 1, 2

1 Monell Chemical Senses Center, Philadelphia, USA; 2 Department of Neuroscience, University of Pennsylvania, Philadelphia, USA; 3 George Washington University, Washington DC, USA; 4 Institute for Diabetes, Obesity and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

Postprandial blood glucose regulation follows a day-night rhythm. In nocturnal rodents, higher glucose tolerance is observed in the beginning of the night, coinciding with the moment of highest food intake. While the brain's biological clock is known to regulate this rhythm, the contribution of peripheral glucose sensing remains unclear. Here, we identify vagal sensory neurons as key mediators of these circadian fluctuations in glucose tolerance. Using live calcium imaging and Fos-TRAP labeling, we found that glucose activates nodose ganglia (NG) neurons in a time-of-the-day-dependent manner, with greater activation at night. Similarly, nighttime glucose administration elicited stronger activation of the dorsovagal complex in the brainstem compared to daytime administration, suggesting that circadian rhythms influence how vagal glucose signals are processed by the brain. Disrupting this rhythmicity by deleting the clock gene Bmal1 in NG neurons abolished the day-night difference in glucose tolerance; instead of the expected nighttime peak, glucose tolerance remained the same regardless of the time of the day. This effect was driven by a reduction in glucose-stimulated insulin secretion at night rather than changes in insulin sensitivity or endogenous glucose production, indicating that circadian control of vagal glucose sensing primarily regulates insulin release. As a potential mechanism, we investigated the neuropeptide cocaine- and amphetamine-regulated transcript (CART), previously shown to be more responsive to nutrient intake at night. We found that NG-specific knockdown of CART impairs glucose-stimulated insulin secretion, suggesting that CART is necessary for the circadian regulation of insulin release at night. Together, these findings establish vagal sensory neurons as a key peripheral component of the circadian regulation of glucose homeostasis. By integrating time-dependent glucose sensing with neuropeptide signaling, vagal sensory neurons dynamically modulate insulin secretion to align with the body's metabolic needs across the day-night cycle.

YIS-3: Divergent modulatory roles of relaxin-3 and oxytocin in the ventral dentate gyrus

Trenk, Aleksandra, 1; Przybylska, Kinga, 1; Stopka, Gabriela, 1; Gugula, Anna, 1; Nogaj, Aleksandra, 1; Czerniak, Gabriela, 1; de Ávila, Camila, 2; Hossain, Mohammed A., 3; Intorcia, Anthony J., 4; Serrano, Geidy E., 4; Beach, Thomas G., 4; Mastroeni, Diego F., 2; Gundlach, Andrew L., 3; Blasiak, Anna, 1

1 Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; 2 ASU-Banner Neurodegenerative Disease Research Center, Biodesign Institute, and School of Life Sciences, Tempe, Arizona, USA; 3 The Florey Institute of Neuroscience and Mental Health, and Florey Department of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia; 4 Neuropathology Laboratory, Banner Sun Health Research Institute, Sun City, Arizona, USA

Oxytocin (OXT) and relaxin-3 (RLN3) are critical neuropeptides regulating the neuronal circuits underlying social, stress, and anxiety-related behaviors. While OXT is well-known for promoting social bonding and mitigating stress, RLN3 action has been associated with heightened anxiety and social avoidance. Despite these contrasting roles, the interaction between OXT and RLN3 signaling systems in relevant brain regions, particularly within the ventral hippocampus dentate gyrus (vDG), remains under-explored. Therefore, we employed multifaceted

approach – including anatomical, molecular and electrophysiological experiments – to elucidate the neuronal circuits controlled by OXT and RLN3 within the vDG and functional interplay of these neuropeptides in the rat brain, with complementary anatomical studies in the human hippocampus.
Viral-based tract-tracing revealed that both the hilus and inner molecular layer of the rat vDG are innervated by RLN3-positive fibers originating from the nucleus incertus (NI), with a predominance of ipsilateral projections. Additionally, HiPlex in situ hybridization (ISH) studies demonstrated that RLN3 receptor (RXFP3) mRNA-expressing cells in the rat vDG co-express markers of inhibitory neurons, such as vesicular GABA transporter (vGAT1) and somatostatin, and identified moderate co-expression of RXFP3 and OXT receptor (OXTR) mRNA.

Electrophysiological recordings using multielectrode arrays revealed that OXT rapidly and directly activates vDG neurons, whereas RLN3 exerts an inhibitory effect. Notably, the modulatory impact of each neuropeptide was attenuated when both were present simultaneously, suggesting a dynamic and reciprocal interaction. Complementary whole-cell patch-clamp recordings confirmed that RLN3 decreases the excitability of vDG granule cells, while OXT enhances the frequency of inhibitory postsynaptic currents.
Results of human hippocampal studies further supported these findings, revealing RLN3-positive fibers and co-localization of RXFP3 with both somatostatin and OXTR mRNA in anterior hippocampal sections.
In summary, our results suggest that vDG serves as an important hub where OXT and RLN3 exert opposing effects on neuronal activity to modulate stress, anxiety, and social behaviors. Importantly, the confirmation of similar organizational patterns in rat and human hippocampal tissue underscores the translational relevance of these findings and raises the possibility that dysregulation of OXT and RLN3 signaling could contribute to the pathophysiology of anxiety disorders.

Supported by National Science Centre Poland: UMO-2023/49/B/NZ4/01885, UMO-2018/30/E/NZ4/00687; MiniGrant 2023 ID.UJ; Bright Focus Foundation – A2021006; Alzheimer's Association – AARFD-22-972099; European Union's Horizon Europe research and innovation program under Marie Sklodowska-Curie grant agreement No 101086247 - PsyCoMed Project.

YIS-4: A genetic strategy to suppress neuropeptide signaling from nociceptors

MacDonald, Donald Iain, 1; Balaji, Rakshita, 1; and Chesler, Alexander T. 1,2
1 National Center for Complementary and Integrative Health, NIH, Bethesda, Maryland, USA; 2 National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland, USA

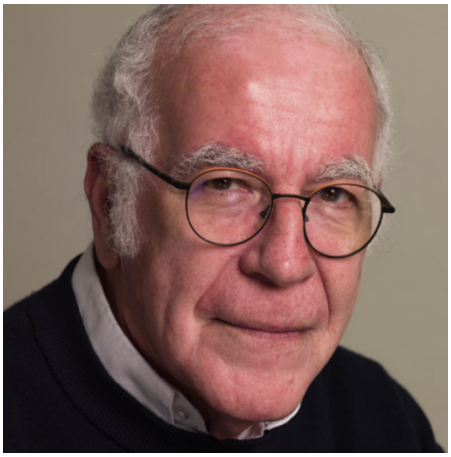
Nociceptor sensory neurons orchestrate behavioral and bodily responses to injury. As well as driving protective behaviors like pain, these neurons can shape inflammation in peripheral tissues. Upon activation, nociceptors release a cocktail of neuropeptides from both their central and peripheral terminals, as well as the fast transmitter glutamate. However, whether the slower neuropeptide signaling from nociceptors plays a driving role in either pain or neuroimmune cross-talk remains unclear. To address this, we developed a genetic strategy to broadly and selectively block neuropeptide signaling from nociceptors, while sparing glutamate transmission. Nociceptor-targeted conditional knockout of the enzyme peptidyl-glycine alpha-amidating monooxygenase (PAM), which directs the final step of neuropeptide biosynthesis, resulted in the complete loss of Substance P and CGRP transmission from nociceptors. Thus, a single enzyme controls nociceptor neuropeptide release and, using these animals, we have now identified essential roles for neuropeptides in controlling pain and inflammation.
Supported by NCCIH DIR, Branco Weiss Fellowship, NIH Office of Autoimmune Disease Research.

RegPep Pioneer-lecture 1

Neuropeptide pioneers

Swaab, Dick

Netherlands Institute for Neuroscience, Department Neuropsychiatric Disorders, Amsterdam, The Netherlands

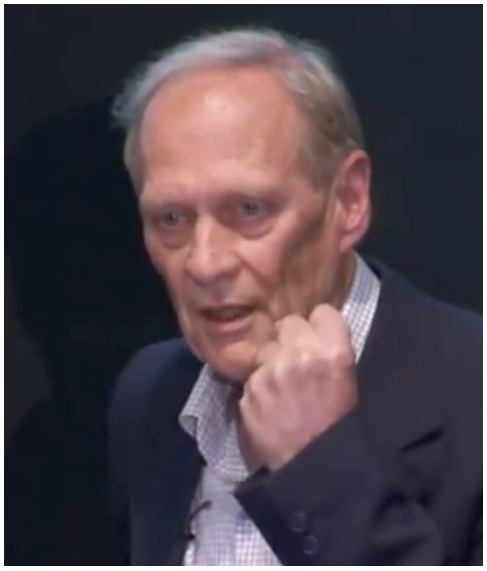


The brain region where peptide research was starting is the hypothalamus, a name coined by Wilhelm His in 1893, but peptides are currently known to regulate processes throughout the body. I will limit myself to my own field, the history of neuropeptides, i.e. regulatory peptide production by neurons and the sensitivity of neurons for these peptides, as well as the dynamic, bidirectional interactions between peptidergic neurons and endocrine glands. These interactions do not only occur through hormones, but are also partly accomplished by the autonomic nervous system that is regulated by the hypothalamus and that innervates the endocrine glands. A special characteristic of the hypothalamus is that it contains neuroendocrine neurons projecting either to the neurohypophysis or to the portal vessels of the anterior lobe of the pituitary in the median eminence, where they release their neuropeptides or other neuroactive compounds into the blood stream, which subsequently act as neurohormones. In the1970s it was found that vasopressin and oxytocin not only are released as hormones in the circulation but that their neurons project to other neurons within and outside the hypothalamus and function as neurotransmitters or neuromodulators that regulate central functions, including the autonomic innervation of all our body organs. Recently magnocellular oxytocin neurons were shown to send not only an axon to the neurohypophysis, but also axon collaterals of the same neuroendocrine neuron to a multitude of brain areas. In this way, the hypothalamus acts as a central integrator for endocrine, autonomic, and higher brain functions. The history of neuroendocrinology can be described from the descriptions of the rete mirabilis in De humani corporis fabrica by Vesalius (1537) to the present. A few examples of such pioneers are: Cajal (1909, innervation of the neurohypophysis), Ott and Scott (1905, milk ejection effect of neurohypophysis), Dale (1906, oxytocic action of neurohypophysis on uterus), Von den Velden/ Farini (1930, posterior lobe extract has antidiuretic effects), Speidel (1917, neurosecretion), E and B Scharrer (1928, hypothalamic neurosecretion), Bargmann (1949, Gomorri staining) Du Vigneaud (1957, elucidating the structure of oxytocin, vasopressin, neurophysin and glycopeptide), Archer (neurophysins: carriers) Verney (1957, osmoreceptors in hypothalamus), Green and Harris (1947, flow direction in portal system), Saffran and Shally, Guillemins and Rosenberg (1955, CRH activity). For central effects of neuropeptides a few examples are: Cushing (1932, parasympathetic central effect of pituitrin), Barry (1954, Gomorri positive ‘synapses’), De Wied (1965, central effects of pituitary hormones), Swaab (1975, vasopressin in SCN) and Buijs, (1979, vasopressine en oxytocine synapses in the brain).

RegPep Pioneer-lecture 2

Gut peptides from the beginning and looking to the future

Furness, John B.
1 Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, Australia; 2 The Florey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia



Gut hormones were the amongst the first hormones to be discovered (if we discount purification of estrogen from urine in ancient China). Although there were early hints in the modern age of scientific discovery (from 1850) for the existence of hormones, it was the discovery of secretin by brother's-in-law Ernest Starling and William Bayliss that provided convincing evidence, and in his Croonian Lecture of June 20 1905 (the 120th anniversary of his lecture being last week) that Starling introduced the new term, hormone, to describe secretin, noting the mechanism of its signaling.

During this spectacular period of discovery, a letter code was introduced to classify gut enteroendocrine cells (EEC), on the assumption of there being one hormone per cell type, except for the L-cell, known to contain PYY and GLP-1. How wrong this was! Studies in the early 2010s clearly showed the error of the one-cell/ one-hormone idea. Complicating the situation even more, there was a highly complex pattern of hormone colocalization (a chemical coding), including regional differences, for example small intestine L-cells containing neurotensin and L-cells in the colon containing INSL5, and differences across the mucosa, from crypt to villus. Investigation of the crypt-villus axis reveals that individual EEC change their hormonal contents through their life cycles. The newly revealed complexity includes that serotonin-containing EEC, formerly thought to be a single cell type, clusters into 14 types when single cell expression is carefully analysed. Similar observations have been made when other hormone types have been focused on.

I will discuss the contemporary challenge of interpreting the language of the GI hormones, released from a broad range of cell types, by many different stimuli, acting on multiple interacting targets and subject to plastic changes.

The author declares no competing interests.

RegPep Pioneers Roundtable

Ruud Buijs, Dave Grattan, Patricia Joseph-Bravo, Dayu Lin, Maurice Manning, Bob Millar

The Regpep Pioneers Roundtable will feature short segments presented by each of five regulatory peptide scientists who in some sense have carried forward the remarkable work of du Vignaud, Harris, Schally and Guillemin and other pioneers to become pioneers themselves in the following remarkable half-century of progress which we are celebrating today as both the 50th anniversary of the society that was inaugurated in 1975 to celebrate progress and further promise in regulatory peptide research and which is today the International Regulatory Peptide Society. **Maurice Manning** will describe the early and continuing importance of solid-phase peptide synthesis and its implications for confirming the biological activities of naturally-occurring peptides and derivatives with experimentally and therapeutically valuable properties of receptor selectivity. **Robert Millar** will add some recollections about the period during which the concept of 'peptide precursors' was developed, and how this allowed the field to think very differently about how peptide synthesis and release are controlled. **Patricia Joseph-Bravo** will discuss the importance of various technologies for detection, measurement, and administration of TRH and other peptides for understanding the physiology and pathophysiology of the hypothalamic-pituitary-thyroid (HPT) axis. **Ruud Buijs** will discuss how anatomical mapping of peptide helped to drive the understanding of their actions. **Dave Grattan** will comment upon the development of the identities of individual regulatory peptides in mammals, both evolutionarily and in the evolution of our knowledge about how and where they act. **Dayu Lin** will comment on the impact of the genetic/optogenetic 'circuit revolution' on progress in regulatory peptide neurobiology and physiology. It is hoped that this Roundtable, as well as input from the floor, will illustrate, and enliven with anecdote, the last 50 years of progress in regulatory peptides and provide some insights into how we got where we are today by overcoming barriers to knowledge through new concepts, and by overcoming barriers to experimentation through new technologies and techniques.

Monday June 23, afternoon

RegPep25 Inaugural Ceremony

Constitution Hall, American University

14:00 - 14:15 Welcome from the AU-CNB and RegPep25 Co-Chairs

14:00 - 15:00 **PL1:** Inaugural Plenary Lecture (PL1):
Michael Greenberg (Harvard University, USA)
Introduced by **Lee Eiden** (RegPep25 Co-Chair)
Sensory experience-dependent regulation of neuropeptides in learning and memory

15:00 - 15:15 Music Interlude 1

15:15 - 16:00 **PL2:** Inaugural Plenary Lecture 2: **Zhou-Feng Chen** (Shenzhen Bay Laboratory, China)
Introduced by **Hugo Tejeda** (IRPS DM)
Neuropeptide coding of itch, pain and touch

16:00 - 16:15 Music Interlude 2

16:15 - 16:50 **PL3:** Lay lecture: **Colin Saldanha** (Director, Center for Neuroscience and Behavior, AU, USA)
Introduced by **Limei Zhang** (RegPep25 Co-Chair)
The challenges of specificity in secreted signaling to integrate physiological processes

16:50 - 17:00 Music postlude

17:00 - 21:00 Group photo
Welcome Reception
Poster viewing (Datablitz presentations: 7:00-9:00pm)
Mary Graydon Center, AU

Inaugural Plenary Lecture (PL1)

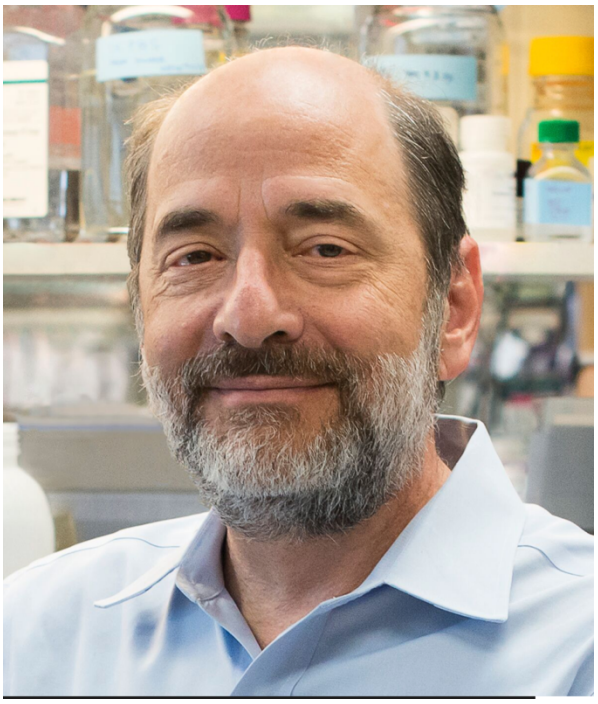
Sensory experience-dependent regulation of neuropeptides in learning and memory

Greenberg, Michael, E.

Deparatment of Neurobiology, Harvard Medical School, Cambridge, Massachusetts, USA

Experience-dependent neuronal activity plays a critical role in shaping the connectivity and function of the central nervous system. The effects of experience are mediated in part by the action of programs of neuronal activity-driven gene expression. Our investigation of these gene expression programs has uncovered important roles in dendritic growth, the maturation and plasticity of excitatory and inhibitory synapses, the process of synaptic pruning, the composition of protein complexes at pre- and post-synaptic sites, and the production of neuropeptides and neuromodulators that control neural circuit development. Moreover, defects in these activity-dependent gene programs contribute to disorders of human cognition. Thus, study of this transcriptional response promises new insights into neuronal plasticity and disease.
The author declares no competing interests

About the speaker



Michael E. Greenberg is the Nathan Marsh Pusey Professor of Neurobiology at Harvard Medical School, renowned for his pioneering research on how sensory experiences regulate gene expression in the brain. He discovered that neuronal stimulation rapidly induces immediate-early genes like *c-fos*, a paradigm-shifting finding that revealed molecular mechanisms linking external stimuli to gene transcription. By elucidating signaling pathways from neurotransmitters to the nucleus, Greenberg's work explained how neural activity sculpts synapses and triggers transcriptional programs underlying brain development, plasticity, and memory formation. His research has also illuminated how experience-dependent regulation of neuropeptides and synaptic proteins supports learning and memory, offering insights into cognitive disorders. An influential leader in neuroscience, Dr. Greenberg is an elected member of the National Academy of Sciences and American Academy of Arts and Sciences. His many honors — including the 2015 **Gruber Prize in Neuroscience** and the prestigious **2023 Brain Prize** — reflect his far-reaching impact on neuroscience and the study of learning and memory.

Plenary Lecture (PL2)

Neuropeptide coding of itch, pain, and touch

Chen, Zhou-Feng

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Differences in the primary afferent fiber size, transduction velocity, and the spinal excitatory interneurons distinguish somatosensory modalities with fast versus slow kinetics. However, a longstanding debate remains on whether the labeled lines (dedicated neural pathways) encode and transmit distinct somatosensory modalities from the periphery to the brain. Itch and pleasant touch, two slow-kinetic modalities with opposing valence, exemplify this dichotomy. In this talk, I will use these modalities to explore the organizing principles underlying the spinal coding and segregation of slow-kinetic somatosensory signals. Our findings reveal that itch and pleasant touch are encoded by modality-specific neuropeptides in primary afferents and relayed via separate labeled lines or microcircuits within the dorsal horn, defined by the non-overlapping GPCR expression profiles. These microcircuits are directly activated by their respective neuropeptides through monosynaptic connections. Thus, the intrinsic properties of slow-kinetic modalities are determined by neuropeptide identity and selective connectivity between primary afferents and spinal microcircuits. While labeled lines in the superficial dorsal horn remain segregated, they can be cross-activated by mechanosensory signals originating in the deep dorsal horn. This crosstalk dynamically shapes sensory perception, emotional states, and behavioral response, offering potential therapeutical avenues to enhance health and well-being by modulating these interactions.

The author declares no competing interests.

About the speaker

Zhou-Feng Chen is Senior Principal Investigator in Neurobiology at the Shenzhen Bay Laboratory, Shenzhen Medical Academy of Research and Translation. A pioneer in somatosensory neuroscience, he discovered the gastrin-releasing peptide receptor (GRPR)—the first itch-specific receptor—and mapped its dedicated spinal circuit, overturning the notion that itch is merely a minor form of pain. He later articulated a "neuropeptide code," showing that modality-selective peptides and their GPCRs—most notably PROK2-PROKR2 for pleasant touch—encode slow-kinetic sensations such as itch, inflammatory pain, and affective touch. Chen also uncovered a non-canonical retina–suprachiasmatic pathway for contagious itch, linking social cues to somatosensory processing. Previously, he founded and directed the Center for the Study of Itch at Washington University School of Medicine, where he was named the Russell D. and Mary B. Shelden Endowed Professor of Anesthesiology.

Lay Lecture (PL3)

The challenges of specificity in secreted signaling to integrate physiological processes

Colin J. Saldanha

Center for Neuroscience & Behavior, American University

The diversity of chemical signaling mechanisms in the brain is a constant source of amazement and curiosity. From neurotransmitters, small molecule hormones, and large proteins; to regulatory elements capable of modifying the synthesis and/or action of all three, these factors impact an impressive range of physiological function dependent upon secreted signaling. This diversity is deepened by the chemical nature of secreted signals. Whereas water soluble peptides need structures or transport mechanisms to cross membranes, lipophilic steroid hormones have the potential of going everywhere. Our lab studies the synthesis of estradiol (E2) by the P450 enzyme aromatase, and focuses on the spatial and temporal specificity of E2 provision in the songbird brain. How is the product of aromatization, a signal that cannot be stored, provided to precise targets and the right time and the right dose. Importantly, this specificity is further challenged by the fact that E2, once a female reproductive hormone, is now known to regulate neurophysiology in both sexes and affects sex behavior, aggression, mood, energy balance, motor function, and learning. We now know that aromatase is expressed in presynaptic boutons and postsynaptic dendrites, suggesting a signaling mechanism that may combine the precision of E2 provision with electrochemical synaptic function. Interestingly, synaptic E2 may support memory function as inhibition of aromatase in the zebra finch hippocampus (HP) impairs spatial memory to the same extent as chemical lesions of the HP, and is restored by concomitant E2 delivery. Further we have learned that reactive astrocytes around brain injuries can rapidly express aromatase and synthesis E2 around the damage thereby controlling several challenging processes associated with traumatic injury. My talk will highlight some of these findings and underscore the need for mechanisms of specificity in secreted signaling.

About the speaker

Colin J. Saldanha is Professor of Neuroscience at American University and Director of the university's Center for Neuroscience & Behavior, where he leads interdisciplinary research and training in brain–behavior relationships. A trail-blazer in neuroendocrinology, he introduced the "synaptocrine" concept after showing that aromatase enables on-site estrogen synthesis within presynaptic boutons and astroglia, delivering hormone signals with exquisite spatial and temporal precision. Work in songbirds and mammalian models from his laboratory has revealed that these locally produced estrogens rapidly modulate learning, memory, neuroprotection, and injury-induced repair.Saldanha's more than 100 publications have been continually supported by NIH and NSF grants, and his dedication to mentoring has earned multiple teaching and advising awards. He is also President-elect (2024–2026) of the Society for Behavioral Neuroendocrinology, underscoring his leadership in the field.

Poster Abstracts

Presented during the Reception June 23, in Mary Graydon Center and exhibited in Constitution Hall June 24-26,

Oxytocin System Modulation via Thyroid Hormone, Probiotics, and Intranasal Oxytocin Improves ASD-Like Deficits from Developmental Pollutant Exposure

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Environmental toxicant exposure may be a contributing factor to the rising prevalence of Autism Spectrum Disorder (ASD). Polybrominated diphenyl ethers (PBDEs) are thyroid hormone (TH)-disrupting chemicals that we have shown produce ASD-like traits and altered social neuropeptide expression in developmentally exposed mice. We hypothesized that since THs regulate oxytocin (OXT), PBDEs may produce ASD-like phenotypes via disruption of the TH system and that restoring TH and OXT would rescue these deficits. C57BL/6N dams were exposed to human-relevant doses of the penta-PBDE mixture, DE-71, at 0.1 mg/kg/d (L-DE-71), 0.4 mg/kg/d (H-DE-71) or vehicle control (VEH/CON) for 10 wks perinatally. A subset of dams received supplementation with levothyroxine (mT4), (8 µg/100 g bw; GD 12-PND 21). On a social novelty preference test, adult male and female L-DE-71 offspring showed no preference for novel over familiar conspecific; mT4 rescued the preference for novelty. DE-71 reduced OXT immunofluorescence IOD and total number of OXT-ergic cells in the paraventricular nucleus (PVH) in male (L- and H-DE-71) and female offspring (L-DE-71) and in L-DE-71 male supraoptic nucleus (SON). mT4 supplementation normalized OXT content and cell number in L-DE-71 female and H-DE-71 male PVH. RT-qPCR showed that sex-dependent gene alterations produced by DE-71 were associated with E2 receptors (Esr1) and TH transporter (Mct8). We tested an OXT- and TH-promoting probiotic Limosilactobacillus reuteri (LR), that improves social behavior in other ASD mouse models and found that LR ameliorated PBDE-induced social and emotional recognition deficits in offspring of DE71 treated dams in a sex dependent manner. Compared to VEH/CON LR treatment ameliorated DE-71-induced deficiency in distinguishing between conspecific-distinct social odors by males and females. Quantification of PBDE- susceptible transcripts using dual immunofluorescence/in situ hybridization showed deregulated thyroid responsive markers on PVH OXTergic neurons. In the LR cohort, DE-71 females but not males showed upregulated TH transporter monocarboxylate transporter 8 (Mct8) and downregulated iodothyronine deiodinase 3 (Dio3) vs VEH/CON. Dio3 expression was normalized in DE-71+LR vs DE-71. Fecal microbiome analysis via 16S rRNA NGS indicated that altered beta diversity in L-DE-71 females was restored with mT4 and LR. Modifying endogenous OXTergic signalling directly with the brain-penetrant melanocortin 4 receptor (MC4R) agonist Ro21-3225 trifluoroacetate salt (7.5 and 15 mg/kg ip) or applying exogenous OXT intranasally (iOXT, 15IU/kg) rescued SNP deficit in FMR1-/- females but not L-DE-71 females. In males, both MC4R agonist and iOXT reversed deficient SNP in L-DE-71, H-DE-71 and FMR1-/-. Exogenous OXT (iOXT; 9 IU/kg) applied during the critical period of development of the OXT system (P7-21), normalized long-term

social recognition memory (SRM) and exaggerated repetitive behavior in males. L-DE-71+iOXT females also displayed reduced PVH OXT immunoreactivity vs. L-DE-71+iSAL. Our results suggest that PBDEs disrupt social behavior and central OXT in an TH-, and sex-dependent manner. Maternal interventions with TH and LR probiotic provide normalization of ASD-relevant behavior and neuromolecular correlates. Collectively, our data elucidates the therapeutic potential of direct and indirect targeting of the oxytocin system to improve ASD-like symptoms in an environmental ASD model.

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Clinical effects of arginine vasopressin on cardiac fibrosis in experimental aortic stenosis in rats

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Vasopressin (AVP) is a neuropeptide with immune stimulatory effects. AVP deficiency decrease immune response including fibrosis. Neuroitermediate pituitary lobectomy (NIL) improve liver and kidney fibrosis. Cardiac fibrosis (CF) involves the expansion of the interstitial space due to the accumulation of extracellular matrix proteins, leading to the replacement of cardiomyocytes with collagen. Myofibroblasts are the primary effectors. Experimental aortic stenosis (EA) in rats induce CF and heart failure caused by pressure and volume overload.
The aim of this experiment is to study the effects of AVP deficiency on reversion of CF and heart failure. Male Wistar rats divided into four groups (8/group): Intact Control (IC), (EA), (NIL), and EA+NIL. EA surgeries were performed at week 0, and LNI and EA+NIL surgeries at week 6 post EA. Evaluated parameters included survival rate, arterial pressure (carotid, femoral, and caudal), cardiac weight, ventricular wall thickness, electrocardiograms (ECG), and exercise tolerance. Statistical analyses included ANOVA, Student's t-test, Mantel-Cox, and Fisher's test as required. The EA+NIL group exhibited a survival rate of 46.15%, whereas the other groups showed survival rates exceeding 90% ($p<0.01$), which was associated with the surgical procedures.

Blood arterial pressure were significantly higher in carotid than in the femoral arteries in both EA and EA+NIL groups ($p<0.002$), confirming adequate EA. NIL induced permanent hypotension in NIL and EA-NIL groups ($p<0.04$). Cardiac weight was significantly higher in the EA group compared to the CI group ($p=0.03$) and the LNI group ($p=0.02$), but no significant differences were noted between EA and EA+LNI groups. Left ventricular wall thickness significantly increased in the EA and EA+NIL groups as compared to the IC and NIL groups ($p<0.004$), this pattern that was also observed in the right ventricle thickness ($p<0.03$). Electrocardiogram revealed a relative risk (RR) of 5.833 for developing cardiac

arrhythmias in the EA group; in contrast, the EA+NIL group showed a RR of 0.667. This finding suggest that AVP deficiency has a protective effect on development of cardiac arrhythmias. In the exercise tolerance test, prior to the NIL surgery the EA and EA+NIL groups demonstrated exercise durations of only 167 seconds, compared to 530 seconds of the IC group ($p<0.0001$); by week 10 (four weeks post-NIL surgery), the EA+NIL group showed a significant recovery, reaching 401 seconds of active exercise ($p<0.0001$). The NIL demonstrated an RR of 0.43 in the EA+NIL group on development of arrhythmias ($p<0.009$).

These findings strongly support that AVP deficiency provides a protective effect against the development of arrhythmias and improves exercise capacity in EA group. These results highlight the protective role of NIL in cardiac fibrosis induced by EA, supported by improvement of key clinical parameters. Although the findings are significant, further validation through additional studies (including molecular and cardiac histopathological analyses) is required. This model opens new perspectives for exploring the pathophysiological mechanisms underlying fibrosis, and the concept of AVP deficiency as a potential therapeutic target.

The authors declare no competing interests.

Chemogenetic inhibition of hypophysiotropic TRH neurons modulates cold stress response and voluntary wheel running in male rats

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The hypophysiotropic thyrotropin-releasing hormone (TRH) neurons, located in the paraventricular nucleus (PVN) of the hypothalamus, play a crucial role in maintaining energy homeostasis and cold adaptation by regulating thyroid hormone levels and directly influencing energy expenditure. When the HPT axis is activated, TRH is released from the PVN, targeting the pituitary gland's thyroid-stimulating hormone (TSH) production. In turn, TSH stimulates the production of thyroid hormones (THs) by the thyroid gland. THs are involved in the response to cold exposure and physical exercise. Acute cold exposure in male rats increases Trh mRNA levels in the PVN and serum TSH, peaking at 1 h. Additionally, voluntary wheel running has been shown to increase Trh mRNA levels in the PVN of exercised male rats in a voluntary wheel running model compared to a pair-fed sedentary group (PF). Furthermore, Trh expression in the PVN, serum TSH, and TH levels positively correlate with voluntarily running distance in the exercise model.

To investigate the effects of inhibiting hypophysiotropic TRH neurons during cold stress and voluntary exercise in Wistar-Han male rats, we employed the chemogenetic tool DREADD. We assessed the functionality of the DREADD in an initial cohort of rats by comparing serum levels of TSH, T3, and T4 in hMDGi4/Cre-TRH rats (n=10) with their control group (Cre-TRH sham-operated) (n=10). Both groups were administered CNO (1 mg/Kg, i.p.) and euthanized 2 hours post-injection. The hMDGi4/Cre-TRH rats showed reduced T3 and TSH levels compared to the Cre-TRH group, while no differences in

T4 levels were observed. After validating the DREADD, we conducted acute cold exposure and voluntary wheel-running experiments with a second cohort of animals.

In the cold exposure experiment, rats were injected with CNO (1 mg/Kg, i.p.) or saline solution (0.15 mL) and exposed to a temperature of 5 °C for 4 hours during their light cycle. Body temperature was recorded each hour using an interscapular implanted microchip, and blood samples were collected before and after one hour of cold exposure to measure TSH concentration. There were no significant differences in body temperature between groups. After one hour of cold exposure, serum TSH levels increased by 1.28 units in Cre-TRH rats (n=6), whereas hMDGi4/Cre-TRH rats (n=11) exhibited a smaller increase of 0.22 units compared to baseline. These results suggest that TRH neuron inhibition blunts the endocrine response to cold.

For the voluntary exercise experiment, hMDGi4/Cre-TRH rats (n=6) and their Cre-TRH controls (n=6) were administered CNO (1 mg/Kg) before access to a running wheel during their dark cycle for 9 days. A pair-fed sedentary group was included for comparison. Body weight, food consumption, and daily running wheel revolutions were measured. Animals were sacrificed three hours after the final exercise session, and parameters related to the HPT axis and exercise were assessed. Notably, the hMDGi4/Cre-TRH group exhibited significantly greater running activity than Cre-TRH rats.
In conclusion, inhibition of hypophysiotropic TRH neurons blunts TSH response to cold without affecting body temperature regulation and enhances voluntary exercise activity, possibly by altering energy balance or motivation for physical exercise.

We thank Rosa María Uribe for teaching the stereotaxic surgery technique used in this study.

The response of the female rat hypothalamus-pituitary-thyroid axis to acute cold exposure is delayed

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Cold exposure triggers thermogenesis in mammals through sympathetic stimulation of brown adipose tissue, skin and muscle and simultaneously, activation of the hypothalamus-pituitary-thyroid (HPT) axis (TRH:TSH:T4/T3). The HPT response is sex dimorphic; Trh mRNA expression increases 2-fold within 1h of cold exposure in the hypothalamic paraventricular nucleus (PVN) of male rats normalizing afterwards, while females do not respond at 1h unless ovariectomized [doi: 10.1530/JOE-14-0593]. Considering sex differences in HPT activity induced by various energy demands [doi: 10.3389/fendo.2021.746924], we studied the dynamics of female response between 0.25 to 5h of exposure at 4°C in Wistar rats using qPCR and ELISA techniques. PVN-Trh expression increased after 4h of cold, when body temperature decreased. As in male, serum [TSH] increased at 15, 30 min and 2h;

serum [T3] augmented after 4-5h. Brown adipose tissue (BAT), the principal thermogenic organ, showed also a delayed increased expression of deiodinase 2 (Dio2) mRNA and uncoupling protein 1. Serum [17β-estradiol] and [prolactin] were stable while serum [progesterone] increased 3-fold from 30 min until 4h [doi:10.1210/jendso/bvae163.2088]. To evaluate central elements that affect HPT axis activity and are modulated by sex hormones, we measured the expression of Dio2 and of TRH-degrading enzyme (Trhde), a peptidase present in tanycytes, in the mediobasal hypothalamus (MBH) [doi: 10.3389/fendo.2019.00401]; Trhde expression decreased after 4h while that of Dio2 increased after 5h (in male, a 2-fold increase in Dio2 expression is seen at 2h). Important modulators of HPT activity include peptides from arcuate neurons such as the orexigenic peptide NPY and the anorexigenic peptides derived from POMC. MBH expression of Npy was enhanced at 2h and then decreased, while that of Pomc decreased at 3 and 4h of cold. In the anterior pituitary, expression of TRH receptor 1 (Trhr) and TSH subunit beta (Tshb) augmented after 3h, that of Trhde and Prl during the first 2 h whereas in male, Tshb levels peaked at 30 min and 2h, and Trhde expression raised in the first 30 min and then diminished, Trhr1 or Prl values did not change. Data confirm a concerted multi-organ response of HPT axis to cold that is sex-dependent; mechanisms involved await elucidation but a plausible candidate involved in sex differences is progesterone whose thermogenic effect might temporarily maintain female body temperature suggesting that hypophysiotropic TRHergic neurons become activated by a drop in body temperature.
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Alpha-MSH regulation of GnRH neuronal activity over development: Dramatic changes in Proestrus females

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Gonadotropin-releasing hormone (GnRH) neurons are essential for fertility, integrating external and internal signals such as metabolic status, to ensure reproductive success. Both overweight and underweight individuals, in rodents and humans, can exhibit reproductive dysfunctions. GnRH neurons express receptors for the anorexigenic proopiomelanocortin, as well as its product, α-melanocyte-stimulating hormone (α-MSH). This suggests a direct link between metabolic function and reproduction. However, how these circuit might interact over development is unclear. To investigate the relationship between α-MSH and GnRH neurons electrophysiology loose-patch clamp recordings of brain slices from prepubertal, pubertal, and adult GnRH-GFP mice were performed in male and female, including 3 stages of the estrous cycle (diestrus, estrus and proestrus). In males and females, □-MSH excited GnRH neurons in prepubertal and pubertal mice. In adult males, most GnRH neurons show no change in activity by □-MSH, while females in either estrus or diestrus show excitation, as detected in younger mice. Notably, in proestrus females a significant inhibition is recorded in GnRH neurons with application of □-MSH. Double labeling for GnRH and α-MSH revealed α-MSH fibers closely juxtaposed to GnRH neurons at all stages. The inhibition observed in proestrus females suggested a third player in this circuit. Inhibition in females during proestrus was found to be via α-MSH activation of nocioceptin/orphanin FQ (OFQ)

inhibitory afferents to GnRH neurons, mediated via opioid receptor-like receptor-1 (ORL-1s). OFQ is known to regulate feeding, increasing food intake via circuits in the arcuate nucleus. However, OFQ also suppresses GnRH neuronal activity, GnRH and LH release. Our results indicate that α-MSH modulates GnRH neuronal activity in a developmental specific manner and that during the LH surge, a time of active mating, metabolic cues can be inhibited on GnRH neurons via the nociception system. Together, these data emphasized a circuit between GnRH neurons, the melanocortin system and the nociception system, linking energy balance and reproduction.

Supported by NINDS – NIH

A BAIAP3 variant in males with pubertal failure: Expression and function in the reproductive axis

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Gonadotropin releasing hormone (GnRH) neuroendocrine cells in the brain release GnRH in a pulsatile fashion at the median eminence to alter hormone secretion in gonadotropes of the pituitary and subsequently the gonads. As such, GnRH cells control reproductive function, being an integral component of the hypothalamic-pituitary-gonadal (HPG) axis. In a previous study, a mutation was identified in the BAIAP3 (brain-specific angiogenesis inhibitor I-associated protein 3) gene in a male patient that had not undergone pubertal changes. BAIAP3 has been implicated in angiogenesis, synapse formation, neuronal development, neurotransmitter release and dysregulation of BAIAP3 has been linked to neuropsychiatric disorders. In neuroendocrine and endocrine cells, BAIAP3 encodes a dense core vesicle (DCV) protein involved in recycling at the trans-Golgi network, consistent with a role in vesicle release. This study examines the expression and function of BAIAP3 in the components of the HPG axis to determine if the mutation in BAIAP3 associated with absence of puberty was in fact causal. BAIAP3 mRNA and protein was found in GnRH cells and during development correlates with maturation of peptide processing and secretion. BAIAP3 mRNA and protein was also found in the testis, localized to mature sperm. Although BAIAP3 was found in the posterior lobe of the pituitary, it was not detected in the gonadotropes in the anterior pituitary. Thus, expression patterns suggest BAIAP3 is involved in two components of the HPG axis, GnRH cells and the gonads. Stimulation of GnRH cells with KCl results in an immediate increase in BAIAP3 expression, consistent with a role in vesicle recycling. Future work will focus on delineating the role of BAIAP3 in spermatogenesis using testis markers. Moreover, the number and function of GnRH cells and spermatogenesis in BAIAP3 KO mice models will be examined. Finally, using an in vitro model known to maintain primary GnRH neuroendocrine cells, we are knocking down BAIAP3 and determining changes in expression of DCV markers, as well as secretion of GnRH. Together these experiments will uncover the role of BAIAP3 in two components of the HPG axis and how disruption of this gene can lead to delayed or absent puberty.

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Differential requirement for glutamatergic and peptidergic neurotransmission at a parabrachioamygdala circuit node mediating behavioral responses to stress and pain

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The parabrachial complex of the brainstem includes the external lateral parabrachial nucleus, which projects to the extended amygdala to mediate the registration of threat, alarm and noxious stimuli for future aversive behavior. A specific glutamatergic parabrachial projection to the capsular nucleus of the central amygdala expresses multiple peptide neuroransmitters, including PACAP, neurotensin and CGRP. Peptidergic involvement within this pathway has been demonstrated for aversive learning, while glutamatergic neurotransmission has been implicated in, but not demonstrated directly in vivo, for pain processing, including that for pain sensitization. Prompted by previous findings that PACAP deletion from these cells results in attenuated behavioral responses to stress, we examined the effects of selective lesioning of PACAP and glutamate neurotransmission from elPBn via unilateral or bilateral injection of AAV-Cre into $PACAP^{fl/fl}$ or $VGluT2^{fl/fl}$ mice, on behavioral responses to restraint stress, pain responsiveness, and pain sensitivity in C57Bl/6 male and female mice. Nocifensive responses to heat, pressure, cold and touch were unaffected in constitutively PACAP-deficient mice, however pain sensitivity responses in mice with nerve injury (sciatic nerve cuffing) in all four sensory modalities were completely eliminated. The constitutive PACAP knockout nocifensive/nociceptive profile was phenocopied in mice in which PACAP expression was absent only from elPBn neurons. $PACAP^{elPBn}$ neurons were equipped with channelrhodopsin, and patency of CNQX-sensitive neurotransmission to central amygdalar PKCδ-positive medium-spiny neurons was tested optogenetically in central amygdala brain slice preparations. Removal of glutamatergic neurotransmission, after elPBn AAV-Cre injection into $VGluT2^{fl/fl}$ mice, also abrogated pain sensitization in mice with nerve injury, without affecting nocifensive or nociceptive responses. In contrast, neither CeC Fos induction nor hypophagia following acute restraint stress were affected by impairment of elPBn glutamatergic neurotransmissioin. Our results suggest that two different types of aversive stimulus response require intact PACAPergic neurotransmission in the elPBn circuit. One of these, pain sensitization, also requires glutamatergic co-transmission. The other, restraint stress-induced hypophagia, does not. Abrogation or preservation of neuronal engagement as monitored by Fos induction consistently mapped to abrogation or preservation of behavioral response upon deletion of either neuropeptide or excitatory vesicular transporter (VGluT2) expression in elPBn neurons. Our results suggest that responses to different modalities of intero- or exteroceptive stimuli, transmsitted within the same neuronal circuit, can be encoded by differential utilization of multiple neurotransmitters within a single circuit node.

A novel PACAPergic projection to the hypothalamus: Prefrontal cortical control of stress responses by the paraventricular nucleus

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The paraventricular nucleus of the hypothalamus (PVN) is the final node in the brain's response to inflammatory, psychogenic and systemic stress through activation of the HPA axis leading to CORT elevation, and triggering behavioral responses to these stressors as well. This occurs via activation of upstream input neurons that converge on the PVN and elicit a cascade of downstream molecular signaling events that control stress responses through CRH neuronal activation. Our laboratory has established that the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is a key mediator of the CRHPVN stress response. Increased expression of Fos, indicating neuronal engagement, and crh mRNA elevation occur in CRHPVN neurons after restraint stress. These responses are abolished in PACAP -/- mice, or mice in which PACAP is deleted only in excitatory forebrain neurons (Camk2a-Cre::PACAPfl/fl), but, surprisingly, not in mice lacking PACAP expression in hypothalamic neurons (Sim1-Cre::PACAPfl/fl mice). Using both anterograde and retrograde tracing techniques, we have now identified a prefrontocortical PACAPergic cell group that forms synapses with CRHPVN neurons. Deletion of PACAP specifically from this neuronal population, in mPFCAAVCre:: PACAPfl/fl mice abrogates both Fos and CRH mRNA induction after restraint stress.

Whether this upstream cortical PACAP projection affects behavioral as well as endocrine stress responses that are mediated through CRHPVN neurons was examined in mice subjected to footshock stress. Footshock (FS)-induced grooming is a self-directed anxiety response to stress that is reported to require engagement of CRHPVN neurons (Bains reference). Accordingly, we examined the PACAP dependence of FS-induced grooming in wild-type, Camk2a-Cre::PACAPfl/fl, mPFCAAVCre::PACAPfl/fl and Sim1-Cre::PACAPfl/fl mice. Deletion of PACAP from the forebrain, or specifically from PACAPmPFC neurons, abolished FS-induced grooming, while abrogation of local PACAP production in hypothalamus was without effect. This modulatory effect of PACAP, like that after restraint stress, spares HPA activation, demonstrating that this more primordial function of CRHPVN neurons is not under cortical PACAPergic control.

We are currently exploring the intracellular signaling pathways that distinguish PACAP-independent control of immediate CRH secretion during stress, from the PACAP-dependent effects on chronic endocrine and acute behavioral stress responses, to determine if the former are mediated by neuronal inputs separate from those mediated by PACAP, or by the same set of neurons but via a different neurotransmitter co-released with PACAP during stress.

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PACAP-stimulated catecholamine synthesis and secretion in NS-1 pheochromocytoma cells

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Acetylcholine (ACh) has been considered the major secretagogue released by the splanchnic nerve, controlling catecholamine secretion from chromaffin cells of the adrenal medulla both basally and under stress. However, pituitary adenylate cyclase-activating polypeptide (PACAP) is also stored and released from splanchnic nerve terminals and is the dominant neurotransmitter for stress-induced catecholamine secretion from the adrenal medulla (Hamelink et al., Proc Natl Acad Sci USA. 2002;99(1):461-6; Smith and Eiden, J Mol Neurosci. 2012;48(2):403-12; Stroth et al., Endocrinology. 2013;154(1):330-9). GLP1 has also been reported as an adrenomedullary secretagogue (González-Santana et al., Cell Rep. 2021;36(8):109609). The adrenal gland releases its entire contents during prolonged splanchnic nerve stimulation, requiring that secretagogues increase compensatory catecholamine synthesis, a process called stimulus-secretion-synthesis coupling. Here, we investigate the potential signaling involved in this process.

We have used cultured Neuroscreen-1 (NS-1) cells, a substrate-adherent PC12 subclone (Emery et al., Sci. Signal. 2013;6:ra51), to examine the signaling pathways leading to PACAP induction of catecholamine (dopamine) biosynthesis and secretion. PACAP, at concentrations as low as 1 nM, causes prompt increases in production of L-DOPA and dopamine (DA) within one hour in NS-1 cells. DA release, as a percentage of total DA content, is increased approximately 6-fold during this time. The increase in L-DOPA production and the secretion of DA is mimicked by the adenylate cyclase activator forskolin and the cell-permeable cAMP analog 8-CPT-cAMP, indicating that biosynthesis and secretion are cAMP-dependent processes, consistent with Gs-coupled adenylate cyclase activation by PACAP. PACAP-dependent synthesis and secretion were unaffected by treatment with the Epac inhibitor ESI-09. The protein kinase A inhibitor H89 blocks PACAP-induced L-DOPA synthesis, suggesting the involvement of PKA in activation of tyrosine hydroxylase, the rate-limiting enzyme for catecholamine biosynthesis. PACAP-induced DA secretion is unaffected by treatment with H89. Secretion is blocked approximately 20% by the L-type calcium channel blocker nifedipine, suggesting that additional calcium channels may be involved in DA exocytotic secretion initiated by PACAP in NS-1 cells. NS-1 cells engineered to express other family B1 GPCRs, including glucagon-like peptide-1 receptor (GLP1R), secretin receptor (SCTR), and vasoactive intestinal peptide receptor type 1 (VPAC1), exhibited PKA-dependent DOPA synthesis and PKA-independent DA secretion when treated with their respective ligands at concentrations of 100 nM. These results suggest a common underlying signaling mechanism for stimulus-secretion-synthesis coupling by family B1 GPCRs.

PACAP-induced secretion of adrenomedullary catecholamines is accompanied by compensatory increase in biosynthesis, shown previously in vivo to be due to increased activity of tyrosine hydroxylase (Hamelink et al., 2002). In NS-1 cells, catecholamine biosynthesis is wholly dependent on PKA, consistent with known mechanisms of TH induction via phosphorylation. However, enhanced secretion is PKA-independent. There are apparently multiple mechanisms for B1 GsPCR-induced secretion: catecholamine secretion from bovine chromaffin cells by exendin-4 is reportedly blocked by 50 nM H89, while secretion from mouse chromaffin cells is reported to be sensitive to inhibition of Epac. Mechanisms of secretion induced by B1 ligands may depend on the complement of voltage-gated calcium channels present in each cell type and the availability of different cAMP-dependent activation pathways.

This work was supported by the NIMH-IRP, Project MH002386.

Converging on PAC1 receptor antagonists through biologically relevant assays, structural biology, and molecular dynamics simulations A comparison of peptide-based and small-molecule ligand results

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The PAC1 receptor for pituitary adenylate cyclase-activating polypeptide (PACAP) is coming into focus as a translationally relevant drug target for neuropsychiatric disorders, atherosclerosis, pain chronification, and protection against neurodegeneration and ischemia. Selective agonists and antagonists for this receptor have long been sought. Given potential PACAP engagement with the related receptors VPAC1 and VPAC2 in vivo, establishing a robust pathway from antagonist screening through demonstration of target engagement is critical for successful PACAP-related drug development. We have developed an in cellula assay for PACAP action in a neuroblastoma cell line expressing endogenous PAC1 and tested a variety of putative small-molecule ligands (SMOLs) and peptides as PAC1 antagonists. These were compared to results from cell lines in which PAC1 is expressed exogenously. We correlated ligand performance with likely mechanisms of action based on structure-based predictions of PAC1 binding. Further, through the development of PAC1 antagonists derived from deletions of the known peptide antagonist PACAP(6–38), we illustrate how structural information about the PACAP binding on the PAC1 receptor deduced from cryo-EM can be leveraged, along with molecular dynamics simulations, to predict candidates for orthosteric binding at PAC1, and to validate existing agonist and antagonist candidates. Finally, we present some recommendations for validation of compounds for in vivo testing, including criteria for target engagement in vivo based on in cellula data. Given that PACAP(6-38) and M65, both peptide-based, are the only validated PAC1 antagonists at this time, development of more potent and selective PAC1 antagonists using a peptide platform seems a fruitful alternative to SMOL-based antagonists.

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Characterizing the dense chemical connectome in Hydra Vulgaris

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Neuroscience is anchored on the paradigm that synaptic transmission in neural circuits generates brain function and behavior, via a specific wiring connectome mediated by excitatory and inhibitory transmission. But an alternative paradigm suggests that neuropeptide-GPCRs interactions form a wireless

"chemical connectome" that could operate in a paracrine manner to generate behavior and brain states. To explore this possibility in basal metazoans, we performed a comprehensive analysis of the cnidarian Hydra vulgaris genome and transcriptome, identifying 61 neuropeptides and 65 neuropeptide-specific G protein-coupled receptors (GPCRs). Distinct profiles of neuropeptide and receptor expression were identified in different neuronal cell types, suggesting specific communication pathways. While neuropeptides appear to have selective expression patterns, GPCRs are more broadly expressed. A diagram of potential interactions between neuropeptides and receptors was computationally derived, deorphanizing receptors with calculated agonist-receptor matching. The topology of this network revealed a dense and distributed connectivity with many neurons being part of the rich club. Ectodermal neurons often had a higher Rich-Club Coefficient and formed a centralized cluster playing a pivotal role in communication within the organism. Specific endodermal neuronal subtypes En3 and En2 act as source nodes, while En1 functions as a sink, mediating directional communication. We also find extensive neuropeptide signaling between the ectoderm and endoderm, supplementing the sparse synoptical connection. This dense neuropeptide network in Hydra can serve as a wireless mechanism for neuronal coordination during complex behaviors. Our findings reveal cell type-specific profiles of receptor and peptide expression, which could facilitate highly specific chemical communication pathways, underscoring the importance of chemical signaling in neuroscience and demonstrating its ancient evolutionary origin.

Neuropeptidergic profile of hypothalamic neurons involved in the control of brown and white adipose tissues in normal and obese rats fed with high energy diet

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Brown adipose tissue (BAT) regulates heat production to maintain body temperature, whereas white adipose tissue (WAT) functions as an energy reserve. Complex brain circuitry controls BAT thermogenesis and WAT lipolysis simultaneously by modulating their sympathetic outflow. Obesity is associated with dramatic changes in BAT and WAT body distribution, and with decreased BAT thermogenic activity. We used a diet-induced obesity (DIO) model in rats to examine the effect of early life obesity on the central circuitry controlling BAT and WAT function. The DIO model generates two divergent phenotypes based on body weight: obese-prone (OP) and obese-resistant (OR) rats. Here, we focus on the effect of early life DIO on hypothalamic nuclei that contain neuropeptides involved in feeding, energy expenditure, and thermogenesis, such as MCH, Orexin, and CART in the lateral hypothalamus (LH), POMC and CART in the Arcuate nucleus (Arc), and CART and Urocortin-1 in the centrally-projecting Edinger-Westphal nucleus (cpEW). Preadolescent rats (28 days-old; n=36) were fed with high energy diet (a.k.a. ice-cream diet; 31.8% kcal from fat and 25.2% kcal from sucrose) for 8-10 weeks, whereas control rats were fed with chow (n=12). Average body weights were 549.8 + 12.7 g (OP), 411.7 + 7.6 g (OR), and 441.3 + 10.5 g (chow). To identify the brain circuit that controls BAT and WAT, rats were injected simultaneously with a retrograde transynaptic pseudorabies virus (PRV) that expresses RFP into inguinal WAT and a PRV expressing GFP into interscapular BAT. Rats were perfused at different survival times (96-124 hours), and brains were processed for immunofluorescence. There were single- and dual-infected neurons in all areas comprising the brain circuitry that controls BAT and WAT. Dual-infected neurons coordinate BAT

and WAT activity simultaneously. At early survival, infected neurons were observed in brain regions involved in sympathetic control. At longer survivals, the infection progressed to hypothalamic areas involved in metabolic control. In LH, around 25-35% of infected neurons contained MCH, whereas around 30% contained Orexin. There were no differences among OP, OR and chow rats. However, there were more infected MCH neurons involved in WAT than BAT control. In Arc, scarce neurons were infected; the % of infected neurons from BAT that contain CART (or POMC) was decreased in OP (21.2 + 1.8) with respect to chow rats (29.8 + 2.6, $p<0.05$). In cpEW, the % of infected neurons from WAT that contain Urocortin-1, a potent anorexigenic neuropeptide, was decreased in OR with respect to chow rats (44.2 + 4.1% vs. 64.1 + 4.1%, $p<0.05$). However, the % of infected neurons from BAT that contain CART was increased in OP (74.4 + 3.4%) with respect to OR (56.5 + 4.3%) and chow rats (63.4 + 2.14%, $p<0.05$) in cpEW. Around ~12% of CART neurons in EWcp were double-infected. These observations support a relevant role of cpEW in the control of BAT and WAT activity. Our results suggest that early life DIO can induce subtle anatomic changes in neuropeptidergic neurons involved in metabolic control and energy expenditure that are anatomically linked to BAT and WAT.

Identification of Cocaine- and Amphetamine-Regulated Transcript (CART) Expression in Waldeyer Marginal Zone Spinothalamic Projection Neurons: Potential Implications for Pain Transmission

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Dorsal spinal cord projection neurons play a crucial role in the transmission of nociceptive signals to the brain. To investigate the molecular profile of second-order spinal nociceptive neurons, we designed a custom Xenium spatial transcriptomic probe set of 300 genes. We identified the expression of neuropeptide cocaine- and amphetamine-regulated transcript (encoded by *CARTPT*) in a distinct population of large dorsal horn "Waldeyer" neurons. These neurons are primarily located in the marginal zone (lamina I) of the dorsal horn and co-express a variety of peptides and receptors related to pain signaling, including *TAC1* (which encodes substance P), cholecystokinin (*CCK*), and the mu-opioid receptor (*OPRM1*). Additionally, these neurons express transcripts associated with glutamatergic transmission, including NMDA receptor subunit 1 (*GRIN1*) and AMPA receptor subunit 4 (*GRIA4*). While most *CARTPT*-expressing neurons are localized to lamina I, some were observed ectopically in the overlying white matter and deeper spinal laminae. These findings were further validated by RNAscope multiplex fluorescence in situ hybridization and immunohistochemical staining, which confirmed that *CARTPT* mRNA and CART protein are expressed in Waldeyer neurons, respectively. Notably, the identification of CARTPT in Waldeyer neurons in this context is human-specific and has not been identified in rodent lamina I neurons. The present study highlights *CARTPT* as a novel transcript potentially involved in spinothalamic communication and pain processing in humans. Future studies will focus on investigating the spatial distribution of CARTPT-containing terminals in the pontine parabrachial nucleus and thalamic medio-dorsal and ventroposterior lateral nuclei to better understand its involvement in CNS nociceptive processing.

Tuesday June 24, Morning

Scientific Session 1 (SS1): Relaxin Family Peptides: From Brain Circuits to Therapeutic Frontiers

Chair: Juan Marugan (NCATS, NIH, USA)

08:00 - 08:05 Introduction

08:05 - 08:30 **Akhter Hossain** (Melbourne, Australia)
RXFP4 and INSL5, physiological roles and therapeutic implications

08:30 - 08:55 **Irina Agoulnik** (Florida, USA)
INSL3 and its receptor RXFP2

08:55 - 09:20 **Francisco Olucha-Bordonau** (Castellón, Spain)
Connectivity of relaxin-3 projections related to cognitive and emotional processes

09:20 - 09:45 **Ross Bathgate** (Australia)
Therapeutic targeting of the relaxin receptor, RXFP1

09:45 - 09:50 General discussion/conclusion

SS1-1: RXFP4 and INSL5, physiological roles and therapeutic possibilities

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The gastrointestinal hormone, insulin-like peptide 5 (INSL5), is located almost exclusively in large intestinal enteroendocrine cells (EEC). One of its functions is to stimulate nerve circuits that increase propulsive activity of the colon through its receptor, the relaxin family peptide 4 receptor (RXFP4). Thus, INSL5 mimetics could possibly be used in the treatment of constipation, a debilitating disorder that is common and at its most severe can damage the large intestine, and even lead to perforation, sepsis and death.

However, INSL5 is a structurally complex two-chain peptide with three disulphide bonds, making it impractical for therapeutic use due to the significant challenges of chemical and recombinant production. Its strong tendency to aggregate further complicates synthesis, requiring laborious multi-step procedures. We aimed to both elucidate how INSL5 exerts its effects and to develop simplified analogues with greater therapeutic potential.

To investigate the mechanisms that link INSL5 to stimulation of propulsion, we have conducted physiological investigations in mice and have determined the localisation of cells expressing Rxfp4, using a Rxfp4 reporter mouse.

In mice treated with the peripherally-restricted mu opioid receptor agonist to slow colorectal motility, thus mimicking opioid-induced constipation, the INSL5 mimetic, INSL5-A13, accelerated colorectal emptying. This anti-constipation effect was not seen when Rxfp4 was knocked out and was reversed by an RXFP4 antagonist that we have developed. Moreover, the effect of INSL5-A13 was prevented by the 5-HT3 receptor antagonist, alosetron. Our localization studies showed that 5-HT containing enteroendocrine cells expressed Rxfp4 and were in close proximity to INSL5 containing EEC. We also showed that 5-HT stimulated the intrinsic nerve circuitry for intestinal propulsion. The INSL5 containing EEC were identified as L-cells that express receptors for bacterial metabolites and are excited when exposed to short-chain fatty acids (SCFA) of bacterial origin. We found that the acceleration of colorectal propulsion by SCFA was reversed by an RXFP4 antagonist. We thus conclude that the physiological role of the INSL5-RXFP4 system is to accelerate colorectal propulsion in the presence of metabolites produced by the fecal microbiome.

We have identified the B chain of INSL5 to be essential for RXFP4 agonism. However, the B chain alone is ineffective in engaging RXFP4, likely because, without the A chain, it fails to adopt a physiologically relevant conformation (α-helical structure). To address this, we explored various peptide-stapling strategies and report here on successful agonism using a truncated B chain, where its α-helical structure was restored through our recently published non-covalent stapling approach. The resulting novel agonist exhibited in vivo efficacy comparable to INSL5-A13 but is substantially simpler and more cost-effective to produce.

In conclusion, we have determined the physiological role and mechanism of the INSL5-RXFP4 system to reverse opioid-induced constipation and have developed simplified, potent derivatives of INSL5 that will be used to investigate therapeutic potential in other constipation phenotypes, including the constipation of Parkinson's Disease and of spinal cord injury.

The authors declare no competing interests.

SS1-2: INSL3 and its receptor RXFP2

Agoulnik, Irina U., 1; Esteban-Lopez, Maria, 2; Wilson, Kenneth J., 3; Myhr, Courtney, 2; Kaftanovskaya, Elena M., 1; Henderson, Mark J., 3; Southall, Noel T., 3; Xu, Xin, 3; Wang, Amy, 3; Hu, Xin, 3; Barnaeva, Elena, 3; Ye, Wenjuan, 3; Ferrer, Marc, 3; Morello, Roy, 4; Marugan, Juan J., 3; Agoulnik, Alexander I., 2

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Insulin-like factor 3 (INSL3) is a peptide hormone produced by testicular Leydig cells in males and, at much lower levels, by ovarian theca cells in females. The relaxin/insulin-like family peptide receptor 2 (RXFP2), a class A G-protein-coupled receptor (GPCR), is the only known receptor for INSL3. This hormone-receptor pair plays a critical role in testicular descent during prenatal development. Male mice lacking INSL3 or RXFP2 exhibit cryptorchidism, with testes retained in a high intra-abdominal position. Genetic studies in cryptorchid patients have identified several mutations in INSL3 and RXFP2, and lower circulating INSL3 levels are commonly observed in orchidectomized and infertile men, as well as individuals with Klinefelter's syndrome and hypogonadotropic hypogonadism. Some reports suggest a role for INSL3/RXFP2 in bone development. Osteoporosis, a chronic bone disease characterized by decreased bone mass and increased fracture risk, is often caused by impaired osteoblast activity. To investigate whether RXFP2 activation can promote bone growth, we screened the NCATS small-molecule library using a cAMP response assay in RXFP2-transfected HEK293T cells. This high-throughput screen identified several low-molecular-weight RXFP2 agonists. Structure-activity relationship (SAR) optimization led to highly potent and selective RXFP2 agonists, confirmed through orthogonal cAMP assays, counter-screens against RXFP1, and comprehensive GPCRome screening using the PRESTO-Tango assay. The selected compounds effectively promoted mineralization in primary human osteoblasts, as demonstrated by increased hydroxyapatite deposition. Mechanistic studies using RXFP2/RXFP1 chimeric receptors established that these compounds act as allosteric agonists, specifically interacting with RXFP2 transmembrane domains. Pharmacokinetic profiling in mice revealed >25% uptake after 24 hours and significant bone exposure. In an efficacy study, 10 mg/kg of the compound delivered by oral gavage to female mice for eight weeks caused marked increase in vertebral trabecular number and thickness, as determined by micro-CT analysis. These findings support the potential of RXFP2 agonists as a novel, cost-effective therapeutic approach for osteoporosis and other bone-loss-related diseases, offering a promising strategy for enhancing bone formation and improving skeletal health.

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SS1-3: Connectivity of relaxin-3 projections related to cognitive and emotional processes

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Cognition and emotion are the result of the process of information throughout circuits involving primarily forebrain anatomic centers. Subcortical afferents to these centers are viewed as modulators of the whole cognitive and emotional functions as part of an arousal mechanism. In this sense, the NI in the pontine tegmentum displays a pattern of ascending connections targeting both cognitive (cored in the hippocampus) and emotional (cored in the amygdala) centers. The NI is characterized by being one of the centers producing the neuropeptide relaxin 3 which is the ligand of the G-protein coupled receptor RXFP3. Either manipulation of the NI or relaxin3/RXFP3 results in modifications in the hippocampal theta rhythm, the response to stress, extinction of fear conditioning or social recognition. These roles are based on specific projections from the NI/relaxin3/rxfp3 to areas involved in a particular function. For example, manipulation of relaxin3/rxfp3 transmission in the retrosplenial cortex impairs extinction of contextual fear conditioning. However, the global pattern of connections has not been analyzed. To address this question, we made retrograde tracer injections in the NI to study the afferences and anterograde tracer injections in areas containing retrograde labeling to confirm these projections and frame them in the global circuit. Retrograde injections in the NI resulted in labeling in the cingulate cortex in a continuum extending from the prelimbic cortex to the retrosplenic cortex, the horizontal diagonal band, preoptic area, lateral hypothalamic, medial division of the lateral habenula, periaqueductal grey, interpeduncular nucleus, median and dorsal raphe. Thus, a first conclusion that can be extracted is that most projections of the NI are in fact bidirectional. Then, anterograde tracer injections in different areas of the cingulate cortex show a pattern of bidirectional projections also between prelimbic, rostral and mid-cingulate and retrosplenial cortex. On the other hand, while retrosplenial cortex is more related to the subiculum and hippocampus, the prelimbic cortex keeps bidirectional connections with the amygdala. Descending projections from both target sequentially the horizontal diagonal band, the supramammillary nucleus, the periaqueductal grey, lateral nuclei of the pons, and the pontine tegmentum including (but not restricted to) the NI. The prelimbic cortex also projected to the periaqueductal grey and pontine tegmentum including the NI. We also observed strong anterograde labeling in the NI from injections in the interopeduncular nuclei, lateral mammillary nucleus, lateral hypothalamic area and habenula. In all cases we observed synaptophysin co-localizing with anterograde labeling in the NI contacting with relaxin3 somata or processes. These results allow us to outline a scheme of connections composed of several subsystem. A) A bidirectional pathway along the gyrus cinguli. B) A system that bidirectional connections between the NI and the retrosplenial cortex which includes the septohippocampal system involved in memory functions. D) the hypothalamic-amygdala loop also projected by the NI and involved in emotional processes. E) The loop between the NI, lateral habenula and interpeduncular nucleus as the core of brainstem processing. F) the main adaptative responses arising from the periaqueductal grey and the lateral hypothalamic area.

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SS1-4: **Therapeutic targeting of the relaxin receptor, RXFP1**

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The peptide relaxin is best known as a pregnancy hormone that plays important roles in cardiovascular function and the development of the reproductive tract throughout pregnancy. The receptor for relaxin is the G protein-coupled (GPCR), relaxin family peptide 1 (RXFP1) receptor. The native relaxin peptide has demonstrated considerable promise as a treatment for cardiovascular disease and fibrosis. While it did not meet long-term primary endpoints in a Phase IIIb trial in acute heart failure (AHF), metanalysis of all the trial data including >11,000 patients with AHF suggested it produces significant reductions in worsening HF and markers of renal function but concluded that long-term outcomes would not be influenced by a 48 hr short-term treatment. Subsequently, numerous pharmaceutical companies have developed both peptide and small molecule long-acting RXFP1 agonists which are being tested in Phase II AHF trials.

RXFP1 is not a classic GPCR target, it contains a large extracellular domain (ECD) with a leucine-rich repeat (LRR) domain and linker region connected to a low-density lipoprotein Class A (LDLa) module. The relaxin B-chain binds with high affinity to both the LRRs and linker and conformational changes in the LDLa+linker induced by relaxin binding are essential for RXFP1 activation. This complex activation mode makes the development of mimetics that exactly mimic the mode of relaxin activation challenging. In line with this, we recently developed a relaxin mimetic peptide, B7-33, and showed it has cell-specific actions. We also have demonstrated that small molecule agonists can show biased signalling properties. A full understanding of the mechanisms of peptide and small molecule activation of RXFP1 requires more structural information.
This presentation will outline the challenges of therapeutic targeting of the relaxin receptor. Further, it will detail efforts to solve the structure of the relaxin-RXFP1 complex and the use of NMR and other advanced biophysical techniques to understand the structural rearrangements associated with LDLa-linker mediated activation.

The authors declare no competing interests.

Scientific Session 2

SS2: Neuropeptidergic Control of Reproduction and Beyond: Insights from Kisspeptin and Oxytocin Circuits

Chair: Mike Lehman (USA)

08:00 - 08:05 Introduction

08:05 - 08:30 **Martin Kelly** (Oregon, USA)

The Role of Hypothalamic Arcuate Kisspeptin Neurons as the Gatekeepers of Fertility

08:30 - 08:55 **Michael Lehman** (Ohio, USA)

KNDy neurons of the hypothalamus: an update of their roles in health and disease

08:55 - 09:20 **Vito Hernandez** (UNAM, Mexico)

Kisspeptin Signaling Beyond Reproduction: Chemoanatomical Characterization and Functional Insights

09:20 - 09:45 **Ryoichi Teruyama** (Louisiana State Univ, Baton Rouge, USA)

Sexually dimorphic oxytocin system in the CNS

09:45 - 09:50 General discussion/conclusion

SS2-1 The role of hypothalamic arcuate kisspeptin neurons as the gatekeepers of fertility

Kelly, Martin J.

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Hypothalamic kisspeptin (Kiss1) neurons and the Kiss1 receptor (Kiss1R) are essential for pubertal development and reproduction. Kisspeptin neurons within the arcuate nucleus of the hypothalamus (Kiss1-ARH) co-express Kiss1, Neurokinin B (NKB) and Dynorphin (Dyn), all of which are down-regulated by high circulating levels (e.g., late follicular levels) of 17β-estradiol (E2). However, Kiss1-ARH neurons also express the vesicular glutamate transporter 2 (vGlut2) and release glutamate. Both Vglut2 expression and glutamate release are increased by E2 in females. Therefore, the peptides and glutamate are differently regulated by E2, which suggests that there is a complex regulation of Kiss1-ARH neurons by E2.

Anatomical studies provided early evidence that Kiss1-ARH neurons can communicate directly with each other. Kiss1-ARH neurons express the NKB receptor, TACR3, as well as the kappa (κ) opioid receptor (KOR), whereas Kiss1R is not expressed by Kiss1-ARH neurons, rendering them unresponsive to kisspeptin. Although single action potential-generated calcium influx is sufficient to trigger the release of classical neurotransmitters such as glutamate, high frequency firing is required for the release of neuropeptides such as kisspeptin, NKB and dynorphin. Indeed, using optogenetics and whole-cell recordings we demonstrated that high-frequency (10-20 Hz) photoactivation (firing) of Kiss1-ARH neurons induces an NKB-mediated slow excitatory postsynaptic potential (sEPSP), which is mediated by the opening of canonical transient receptor potential 5 (TRCP5) channels. CRISPR/SaCas9 mutagenesis of Trpc5 in Kiss1-ARH neurons abrogates the sEPSP. The release of NKB is limited by co-released dynorphin, which activates presynaptic KOR's to inhibit further NKB release. The actions of these two peptides cause synchronized firing of Kiss1-ARH neurons, whereas kisspeptin and glutamate are the main output signals. The sEPSP is similar to the "plateau potential" that has been described in hippocampal and cortical neurons. Many CNS neurons, including Kiss1-ARH neurons, express the biophysical properties (e.g., TRPC5 channels) that allow them to continue to fire persistently even after a triggering synaptic event has subsided. Although the expression of Trpc5 in Kiss1-ARH neurons is downregulated by E2, the intrinsic excitability of Kiss1-ARH neurons is augmented by the increased expression of Cav3.1, T-type calcium (Cacna1g) channel and Hyperpolarization-activated, Cyclic Nucleotide Gated (Hcn1, Hcn2) channels. Burst firing, which efficiently releases fast amino acid transmitters like glutamate, is generated primarily by the T-type calcium channel current and the rhythmicity of this burst firing is dependent on the h-current.

As proof of principle, we demonstrated that optogenetic stimulation of Kiss1-ARH neurons releases glutamate and excites anteroventral periventricular and periventricular nucleus Kiss1 (Kiss1-AVPV/PeN) neurons via activation of both ionotropic and metabotropic glutamate receptors. Moreover, CRISPR/SaCas9 mutagenesis of Vglut2 in Kiss1-ARH neurons abolished glutamatergic neurotransmission, which significantly reduced the overall glutamatergic input to Kiss1-AVPV/PeN

neurons. In vivo, the mutagenesis of Vglut2 in Kiss1-ARH neurons abrogated the E2-induced LH surge and reduced the formation of corpus lutea, indicative of a reduced ovulatory drive in these Vglut2 mutated Kiss1-ARH mice. Therefore, Kiss1-ARH neurons appear to play a critical role not only in "pulse generation" but also in augmenting the GnRH surge through switching from peptidergic to glutamatergic neurotransmission to excite Kiss1-AVPV/PeN neurons.

Collaborators: Jian Qiu, Martha A. Bosch, Oline K. Ronnekleiv (OHSU); Larry S. Zweifel (U. Washington); Rajae Talbi, Elizabeth Medve, Victor M. Navarro (Brigham and Women's Hospital, Harvard). Funding was provided by NIH grants: R01-DK68098 (MJK & OKR), R01-HD090151, R01-HD099084 and R01-DK133760 (VMN); P30-MH048736 and R01-MH104450 (LSZ)

SS2-2 **KNDy neurons of the hypothalamus: an update of their roles in health and disease**

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Identification in 2007 of a subpopulation of neurons in the arcuate nucleus of the hypothalamus, that co-expressed the three neuropeptide, kisspeptin, neurokinin B, and dynorphin (termed "KNDy" neurons) was a pivotal discovery leading to the hypothesis that these cells were the long-sought pulse generator responsible for the episodic release of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) that is critical for fertility. Experiments over the years since have provided compelling evidence that KNDy neurons serve as the GnRH pulse generator in a wide range of mammals and play a critical role in development and puberty as well as adult reproductive function. We will review this evidence, as well as the relatively rapid translation of the basic neurobiology of KNDy cells into new clinical treatments for common reproductive disorders, such as polycystic ovarian syndrome (PCOS) and post-menopausal hot flushes. We will also highlight the way in which newly developed technologies, such as optical tissue clearing, light sheet microscopy, transgenic-mediated multi-synaptic tracing, and *in vivo* calcium imaging contributed to this knowledge. Finally, we will review recent modifications of the original KNDy hypothesis along with evidence of an expanded role for KNDy, and adjacent ARC, neurons in reproduction and other homeostatic functions.
Supported by NIH R01 HD39916, R01 HD114782.

SS2-3 Kisspeptin signaling beyond reproduction: Chemoanatomical characterization and functional insights

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Kisspeptin (KP) is a well-established regulator of reproductive function, but emerging evidence suggests its role extends beyond the hypothalamic-pituitary-gonadal axis. In this talk, I will present recent chemoanatomical findings suggest a new neurobiological landscape for kisspeptinergic signaling. Using a comprehensive approach that combines immunohistochemistry and RNAscope in situ hybridization, we characterized the distribution of KP fibers and Kiss1 receptor (Kiss1r) expression across 118 regions of the rodent brain. Notably, KP-immunoreactive fibers and Kiss1r expression were identified in extra-hypothalamic structures involved in sensory integration, arousal, and cognitive function, including the thalamus, hippocampus, septal nuclei, and brainstem.

Additionally, we identified seven distinct kisspeptinergic neuronal populations in the mouse brain, including two novel groups in the ventral premammillary nucleus and nucleus of the solitary tract. Chemotyping analyses revealed diverse neurotransmitter co-expression patterns, including GABAergic and glutamatergic markers, as well as sex steroid receptors, suggesting complex regulatory mechanisms. Gonadectomy-induced alterations in Kiss1 expression further indicate dynamic endocrine modulation of these populations.

Together, these findings provide a refined understanding of the kisspeptinergic system as a neuromodulatory network with potential implications for sensory and behavioral state control. By extending beyond traditional reproductive paradigms, this research opens new avenues for investigating kisspeptin's role in broader neurophysiological processes.

Supported by CONAHCYT CF-2023-G-243, PASPA-UNAM scholarship, Fulbright-Garcia Robles Fellowship

SS2-4 **Sexually dimorphic oxytocin system in the central nervous system**

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The supraoptic nucleus (SON) and paraventricular nucleus (PVN) in the hypothalamus contain magnocellular neurosecretory cells (MNCs) that synthesize the neurohypophysial hormone, oxytocin. The MNCs send long axonal projections into the neurohypophysis where oxytocin is released into the general circulation in response to physiological demands. Oxytocin is known for its critical role in parturition and lactation. Subsets of oxytocin MNCs collaterally projects to both the neurohypophysis and the extrahypothalamic regions. When oxytocin is released within the brain, oxytocin modulates many aspects of social behaviors, such as social recognition, pair bonding, and maternal behavior. Oxytocin influences behaviors by binding to oxytocin receptors (OXTRs) that are widely distributed in various parts of the brain. Using the OXTR-Venus mice, we discovered female specific expression of OXTR in the anteroventral periventricular nucleus (AVPV), the perinuclear zone (PNZ) of the SON, and the retina. The expression of OXTR in these areas are estrogen dependent, since ovariectomy blocked the expression of OXTR-Venus whereas estradiol implant restored the expression. The expression of OXTRs in the AVPV increased significantly during postpartum period, and chemogenetic inactivation of the OXTR cells blocked pup retrieval and nest-building behavior without significantly affecting nursing behavior such as feeding pups. Pup retrieval and nest building behaviors are voluntary acts driven by attraction toward pup-related stimuli and are more indicative of maternal motivation. In contrast, nursing behaviors are likely elicited by proximal pup stimuli that are regulated largely by brainstem mechanisms and are indicative of a more reflexive maternal behavior. Postpartum female mice display a significantly increased motivation to interact with pups compared to non-postpartum females. Thus, the increased expression of OXTR in the AVPV of postpartum female may be the neurobiological basis for the increased maternal motivation. In any case, the findings suggest that the neural activity of AVPV-OXTR cells is essential for the induction of maternal motivation. Our previous study also found that approximately 30% of OXTR cells in the AVPV were tyrosine hydroxylase immunoreactive (TH+) putative dopaminergic cells. Thus, there are at least two types of OXTR cells in the AVPV. Our ongoing study found that TH+ OXTR cells exhibit intrinsic tonic bursting without any synaptic inputs, whereas non-TH+ OXTR cells exhibit continuous or phasic bursting firing pattern. Regardless of the cell types, all OXTR cells responded to the bath application of the selective OXTR agonist, [Thr4, Gly7]-oxytocin (TGOT), by depolarization and increasing firing frequency. In addition to the SON and PVN in the hypothalamus, oxytocin MNCs were identified in the preoptic area (POA) where the anterior commissural nucleus (ACN) and AVPV comprise the third largest population of oxytocin MNCs. Our immunohistochemistry showed that the OXTR neurons are intermingled with oxytocin immunoreactive neurons and processes in the AVPV. The close proximity of OXTR cells and oxytocin cells in the AVPV lead us to hypothesize that the somatodendritic local release of oxytocin within the AVPV modifies neuronal activity of the OXTR cells to facilitate maternal motivation, while their oxytocin release from the neurohypophysis coordinates peripheral oxytocin-mediated physiology.

Scientific Session 3

SS3: Neuropeptides and Social Motivation: Circuits Shaping Sex Behavior, and Choice

Chair: David Keller (Germany)

10:00 - 10:05	Introduction
10:05 - 10:30	**Aras Petrulis** (GSU, USA)
	Vasopressin in the lateral septum drives sex-specific social interest
10:30 - 10:55	**Mario Gil (**Texas, USA)
	Social and Sexual Behaviors Regulated by AVP and OT in Rodent Forebrain and Midbrain
10:55 - 11:20	**David Keller** (University of Cologne, Germany)
	Neuronal populations in the lateral septum regulate sex-dependent social interactions and feeding behavior
11:20 - 11:45	**Ki Goosens** (Icahn School of Medicine at Mount Sinai, USA)
	Regulation of complex decision-making by ghrelin
11:45 - 11:50	General discussion/conclusion

SS3-1: Vasopressin in the lateral septum drives sex-specific social interest

Petrulis, Aras

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While vasopressin (AVP) has long been implicated in social behavior, the exact anatomical substrate of AVP's control of social behavior is unclear. We have recently demonstrated that the AVP-expressing cells in the bed nucleus of the stria terminalis (BNST), the major source of sexually-differentiated AVP in the brain, primarily drive male investigation of other males in mice. The effect of BNST AVP cells on male-male social interest is, in part, due to action on the lateral septum (LS), a major output of BNST AVP cells and which strongly expresses vasopressin 1a receptor (V1aR). Stimulation of BNST AVP cells terminals in the LS increased male, but not female, social investigation, effects that could be blocked by V1aR antagonist. Stimulation of BNST AVP terminals ex vivo phasically increased, then decreased LS cell activity (V1aR-dependently), mimicking the timeline of in vivo increase in social investigation, suggesting that AVP-V1aR mediated inhibition of LS permits high levels of social investigation in males. Preliminary findings from immediate-early gene expression patterns and in vivo calcium dynamics in response to social interactions support this hypothesis. We have also found that V1aR+ LS cells receive their strongest inputs from ventral hippocampal and project primarily to the diagonal band of Broca, lateral habenula, lateral hypothalamus, and supramammillary nucleus, all areas that also receive steroid-sensitive, sex-different AVP fibers and express substantial amounts of V1aR. Consequently, BNST AVP cells may modulate contextual information (from hippocampus) to alter activity of an interconnected AVP-sensitive circuit and ultimately facilitate sex-specific social approach and investigation.

Supported by NIMH

SS3-2 Regulation of social and sexual behaviors by neurohypophysial hormones in the rodent forebrain and midbrain

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The neurohypophysial hormones oxytocin (OT) and arginine-vasopressin (AVP) play a major role in the control of reproductive function, sexual behavior, and social behavior. Others have reported a positive correlation between OT mRNA levels and individual differences in male sexual behavior, and our work also confirms the relationship between OT and sexual behavior. That is, sexually efficient males have lower levels of OT receptor (OTR) binding in the rostral MPOA of the hypothalamus, a major integrative site for sexual behavior, compared to inefficient animals, perhaps due to downregulation of OTRs in response to higher OT levels in efficient males. Acute sexual experience stimulates OTR mRNA expression in the MPOA, with the highest levels being observed in first-time copulators, whereas repeated sexual experience is associated with higher OTR protein levels in the MPOA. Intra-MPOA

injection of OT facilitates copulation and improves sexual efficiency, whereas MPOA injection of an OT antagonist (OTA) inhibits certain aspects of copulation. Thus, our results show that OT in the MPOA facilitates copulation in both sexually naive and experienced males, and some of the behavioral effects of OT are mediated by the OTR. Although the behavioral effects of neurohypophysial hormones in the hypothalamus have been well characterized, more work is needed to elucidate interactions between these hypothalamic peptides and the midbrain component of the mesocorticolimbic system, which plays a role in motivation and reward. Our preliminary data suggest that OT in the rat hypothalamus modulates dopamine activity in the mesocorticolimbic system, and this modulation is associated with OT-induced changes in sexual behavior. In the Syrian hamster model, our findings, as well as reports from other research groups, indicate that both OT and AVP act in the hypothalamus and ventral midbrain to regulate social behavior in a sex- and experience-dependent manner. Collectively, these data support the guiding hypothesis that neurohypophysial peptides regulate sexual and social behaviors by acting directly in the hypothalamus and modulating activity of the mesocorticolimbic system. Moreover, OT and AVP underlie individual differences in behavior and may have therapeutic potential for neuropsychiatric disorders and health issues that are associated with social impairment and/or psychosocial stress.

Supported by NIH grant #1K01HL145339-01A1 (PI: M. Gil)

SS3-3 Neuronal populations in the lateral septum regulate sex-dependent social interactions and feeding behavior

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Social behaviors, whether conflictual or cooperative, are essential for survival and reproduction. However, the neural circuit mechanisms that regulate different social behaviors are still not well understood. Additionally, there is limited knowledge about how the brain processes decisions when faced with competing stimuli that drive mutually exclusive behaviors. The lateral septum (LS), a key brain region, plays a role in regulating aggression and feeding behaviors through its connections with the hypothalamus, prefrontal cortex, and hippocampus. We previously showed that somatostatin-expressing (Sst) neurons in the LS promote food-seeking (Carus-Cadavieco et al., Nature 2017). Here we investigated functions of two cell populations in the LS, Sst- and neurotensin-expressing (Nts) cells, in sex-dependent social- and feeding-related behaviors.

We combined opto-, chemogenetics and calcium imaging in freely behaving mice, to characterize to role of Sst- and Nts -expressing cells in social- and feeding-related behaviors.

Based on the calcium imaging data, we clustered Nts and Sst cells according to their distinct coding patterns for food and social interactions, as well as their responses to same-sex and opposite-sex intruders.

Optogenetic activation of Nts cells resulted in increased social interaction. At the same time, opto- or chemogenetic activation of Nts cells in the LS decreased food intake.

Conversely, optogenetic activation of Sst cells in the LS reduced social interactions. Both Nts and Sst cells exhibited positional firing, whereas Sst cells had higher place field stability and mutual information. Optostimulation of hippocampal inputs to the LS- Nts and LS-Sst cells changed activity of those cell populations.
In summary, our findings indicate that Sst- and Nts -expressing cell populations in the LS work in a complementary manner to regulate various aspects of innate behaviors.
Supported by Alexander von Humboldt Foundation (to D.K.), DFG (SFB1089 and EXC2030-CECAD to T.K., 233886668-GRK1960 to F.S.).

SS3-4 Regulation of complex decision-making by ghrelin

Salicido, AA, 1; Reyes, NF, 1; Macias, AY, 1; Batson, SA, 1; Beck, DW, 2; Friedman, A, 1, 2; Goosens, KA, 3
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Our daily lives are characterized by constant decisions, motivated by pursuit of reward and avoidance of costs. However, it is rare that a decision involves reward without cost, or cost without reward. Instead, most value-based decision-making (DM) reflects a complex integration of costs and benefits together. Stress can alter the decisions that we make by changing how we value and integrate costs and rewards. For example, when stressed, we might be more likely to eat junk food (a palatable reward) between meals despite it contributing to tooth decay (a potential cost). Published work shows that the striosomes of the dorsomedial striatum (DMS) play a critical role in cost-benefit decision-making (DM), where choices lead to both rewards and costs. In general, rewards associated with low costs are pursued more frequently than rewards paired with high costs. However, several factors influence an individual's willingness to pursue offers in which high rewards come with high costs, a so-called 'risky' decision. Our work shows that chronic stress exposure increases the pursuit of risky offers in rodents, but the mechanisms of this change in DM behavior are unknown.
Our published work shows that acyl-ghrelin (aGHR) is elevated by chronic stress in rodents and humans. The sole receptor for aGHR (GHSR) is found in brain circuits that process cost and reward, including striatum. Here, I will describe our recent work linking elevated aGHR signaling in the striatum to stress-related pursuit of risky offers in a rodent cost-benefit decision-making task. I will also talk about our efforts to link the elevated aGHR observed in post-traumatic stress disorder to altered cost-benefit decision-making in humans.
The authors declare no competing interests.

Scientific Session 4

SS4: Neuropeptides and Emotional Brain States: From Circuit Salience to Synaptic Precision

Chair: Francesco Ferraguti (Austria/Italy)

10:00 - 10:05	Introduction
10:05 - 10:30	**Norbert Hajos** (IN, USA) ***VIP-containing midbrain input to central amygdala controls contextual fear memory formation***
10:30 - 10:55	**Claudia Schmuckermair** (U. Innsbruck, Austria) ***Control of salience detection by VIPergic insular interneurons***
10:55 - 11:20	**Limei Zhang** (UNAM, Mexico) ***Neuropeptide in fast and slow synaptic transmission: A discovery of a calyx of Held-like synapse in the rodent forebrain***
11:20 - 11:45	**Anna Blasiak** (Jagiellonian University, Krakow, Poland) ***Interplay of relaxin-3 and oxytocin systems in shaping ventral hippocampus neuronal activity - possible involvement in anxiety control in rat and human***
11:45 - 11:50	General discussion/conclusion

SS4-1 VIP-containing midbrain input to extended amygdala controls contextual fear memory formation

Müller, Kinga, 1,2; Bruzsik, Bíborka, 1,2,3; Karlócai, Mária Rita, 3; Barabás, Bence, 2,3; Nagy, Gergő A., 1,2; Rovira-Esteban, Laura, 1; Fekete, Zsuzsanna, 1,2; Paradiso, Enrica, 4; Blasco-Ibáñez, José-Miguel, 5; Veres, Judit M., 1; Reéb, Zsófia, 1,6; Weisz, Filippo, 1; Papp, Orsolya I., 1; Pardo-Bellver, Cecília, 1,5; , Magyar, Dániel L., 2,3; Tóth, Máté, 1; Szocsics, Péter, 1; Mihály Salamonné, Orsolya, 7; Ferraguti, Francesco, 4; Mikics, Éva, 1; Hajos, Norbert, 1,3,8

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Remembering dangerous contexts is essential for survival, yet the neural circuits and mechanisms underlying contextual fear memory formation remain incompletely understood. Recently we identified a population of neurons expressing vasoactive intestinal polypeptide (VIP) in the ventral periaqueductal gray and dorsal raphe nucleus (vPAG/DRN) of mice. These midbrain VIP neurons project to the central nucleus of the amygdala and the bed nucleus of the stria terminalis - two key components of the extended amygdala (EA) involved in fear processing. Importantly, VIP-containing neurons and axon terminals were also observed in the PAG and EA of postmortem human brain tissue, suggesting this peptidergic pathway is evolutionarily conserved. Inhibition of midbrain VIP neuronal function in mice selectively impaired contextual, but not cued, fear memory formation. These neurons were activated by foot shocks, formed reciprocal connections with the EA, and received input from brain regions involved in threat detection. Using in vitro electrophysiology and optogenetics, we found that midbrain VIP afferents converge onto EA neurons that also receive projections from the ventral hippocampus (vHC) - a region known to convey contextual information to its downstream regions. In brain slice preparations, subsequent activation of VIP and vHC inputs resulted in enhanced synaptic transmission at vHC axons. Furthermore, contextual fear conditioning led to potentiation of synaptic responses at vHC synapses in EA neurons. This learning-induced synaptic plasticity was dependent on the activity of midbrain VIP neurons. Notably, the strength of vHC synaptic responses after fear conditioning positively correlated with freezing behavior during memory recall. Together, our findings reveal a novel role for vPAG/DRN VIP neurons in regulating contextual fear memory formation by potentiating vHC synaptic transmission in EA neurons, uncovering a crucial mechanism for context-dependent threat learning.

SS4-2 Control of salience detection by VIP-containing insular interneurons

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Aberrant functional connectivity between the salience network and the default mode network is one of the most consistent anomalies reported in autism spectrum disorder (ASD) (Uddin et al., 2015). Our data suggest that vasoactive intestinal polypeptide (VIP)-expressing interneurons (VIP-INs) in the anterior insular cortex (aIC) play a crucial role in salience detection and gating sensory stimuli to adaptively shape behavior (Ramos-Prats et al., 2022). The aIC, along with the anterior cingulate cortex, forms the salience network, which filters the most relevant internal and external stimuli to guide behavior.
We hypothesize that impaired VIP-IN function in the aIC disrupts salience encoding, leading to altered sensory processing and contributing to ASD symptomatology. To test this, we generated a mouse model with selective deletion of an ASD-associated gene in VIP-INs. We employ a classical auditory oddball paradigm in head-fixed mice, where a low-probability oddball tone (10%) is embedded within a high-probability standard tone (90%). Using dual-color calcium imaging, we simultaneously monitor principal neuron and VIP-IN activity in the aIC while tracking arousal states via pupil size fluctuations. Preliminary findings will be presented at the meeting.
Supported by the Austrian Science Fund FWF FG18-B.

SS4-3 Neuropeptide in fast and slow synaptic transmission: A discovery of a calyx of Held-like synapse in the rodent forebrain

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Fast and reliable excitatory transmission is essential for neural circuits for rapid sensory processing. The calyx of Held is a giant excitatory cup-like axo-somatic synapse in the auditory brainstem and the only synapse of its kind

described in the central nervous system. Here, using PACAP immunohistochemistry combined with confocal and tomographic electron microscopy, we report the discovery of a morphologically similar calyx-like synapse in the extended amygdala of rodents that exhibits unique neurochemical features. This structure forms enveloping axo-somatic terminals with mixed glutamatergic and cholinergic identities, co-expressing VGluT1, VGluT2, VAChT, and the neuropeptides PACAP, CGRP, and neurotensin, along with calretinin in the presynaptic compartment. The postsynaptic targets are a distinct subset of PKCδ-expressing neurons that co-express the synaptic adhesion molecule GluD1. Strikingly, GluD1 immunolabeling is concentrated specifically at axo-somatic contact sites apposed to VAChT+ synaptic specifications, but absent at postsynaptic density (PSD) of conventional type I synaptic specifications, within calyceal terminals, suggesting a specialized molecular architecture for them. Our findings reveal a previously unknown calyx-like synapse in the forebrain, exhibiting a unique convergence of fast and modulatory transmission with implications for transmission fidelity within an emotional-viscerosensory circuit.

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SS4-3 Interplay of relaxin-3 and oxytocin systems in shaping ventral hippocampus neuronal activity - possible involvement in anxiety control in rat and human

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Neuropeptides are key modulators of neural circuits, acting through direct effects on neuronal excitability and modulation of classical neurotransmission. Among them, oxytocin (OXT) and relaxin-3 (RLN3) have emerged as critical regulators of stress, anxiety, and social behaviors. While OXT, primarily released from the hypothalamus, is well known for its anxiolytic and prosocial effects, RLN3—synthesized in the brainstem nucleus incertus (NI)—is associated with heightened anxiety and social avoidance. Despite their contrasting roles, growing evidence suggests an intricate interplay between these neuropeptide systems, particularly in the ventral hippocampus, a structure implicated in emotional processing. However, the mechanisms underlying this interaction remain poorly understood.

An important recent discovery is the potent inhibitory influence of RLN3 on magnocellular neurons of the paraventricular hypothalamic nucleus, a major source of oxytocinergic projections. Moreover, OXT reciprocally modulates NI neurons, highlighting a mutual feedback mechanism. Beyond this reciprocal control, OXT and RLN3 networks interact within target structures such as the ventral hippocampus dentate gyrus (vDG), where they converge onto a shared population of interneurons.

Notably, at the cellular level, OXT and RLN3 exert opposing effects. OXT, acting via the Gq-coupled OXTR, induces excitatory effects mainly by closing M-type K+ channels and depolarizing neurons, whereas RLN3, through the Gi/o-coupled RXFP3, exerts inhibitory effects via M-channel opening and hyperpolarization. Electrophysiological recordings in vDG interneurons confirmed this antagonistic interaction: OXT increased neuronal excitability, while RLN3 suppressed it. Interestingly, simultaneous activation of both neuropeptide

systems led to mutual attenuation of their respective effects, adding an additional layer of complexity to their regulatory influence on hippocampal circuits.

Beyond rodent models, our human hippocampal studies revealed the presence of RLN3-positive fibers in the anterior hippocampus, as well as co-localization of RXFP3 and OXTR mRNA in hippocampal interneurons, indicating the conservation of this neuropeptide interaction across species. These findings suggest that the balance between OXT and RLN3 signaling in the ventral/anterior hippocampus is an important factor in regulating emotional and cognitive processes, with potential implications for stress-related disorders.

Our study provides novel insights into the integrative role of OXT and RLN3 in shaping hippocampal network dynamics. By revealing a shared circuit in the ventral/anterior hippocampus, where these neuropeptides exert reciprocal and opposing influences, our findings highlight the potential involvement of OXT-RLN3 interactions in anxiety regulation. Furthermore, the similarity in rat and human hippocampal organization of the studied neuropeptide systems confirms the conserved nature of these neuronal circuits and underscores the potential of animal studies to provide valuable insights for developing new therapeutic strategies targeting neuropeptide signaling.

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Tuesday June 24, Afternoon

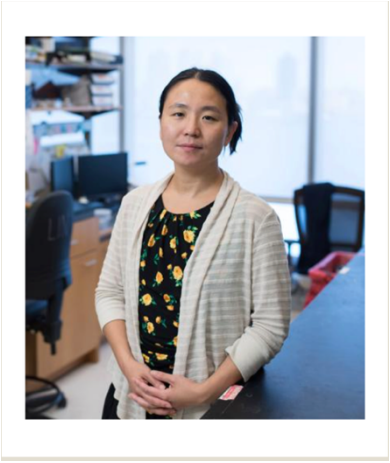
PL4: Plenary Lecture: The neural mechanisms underlying the rise and fall of maternal aggression

13:00 - 13:45 **Dayu Lin** (New York University, USA)
introduced by **Dave Grattan**

Neuroscience Institute and Department of Neuroscience and Physiology, New York University Grossman School of Medicine, New York, New York, USA

To protect the helpless young, lactating females dramatically increase aggression towards intruders, known as maternal aggression. However, attack is costly and risky. When pups no longer exist, maternal aggression rapidly declines. In this talk, I will share our recent study that identified the critical role of the pathway from posterior amygdala to the ventrolateral part of ventromedial hypothalamus in maternal aggression and the multi-form plasticity in this pathway supporting the rise and fall of maternal aggression. *The author declares no competing interests.*

About the speaker



Dayu Lin, Ph.D., is Professor of Psychiatry, Neuroscience, and Physiology at the New York University Grossman School of Medicine, where she leads the Lin Laboratory for Social Behavior Neuroscience. A native of Shanghai, she completed her B.S. in Biology at Fudan University, earned her Ph.D. in Neurobiology at Duke University under Lawrence Katz, and conducted post-doctoral research with David J. Anderson at the California Institute of Technology before establishing her independent group at NYU in 2010.

Dr Lin's work dissects the neural logic of innate social behaviors, combining optogenetics, in vivo calcium imaging, and single-cell transcriptomics to map how genetically defined hypothalamic and amygdalar circuits generate aggression, defense, and sexual behaviors. She and her colleagues identified estrogen-receptor-α–expressing ($Esr1^{+}$) neurons in the ventrolateral ventromedial hypothalamus (VMHvl) as a dedicated "attack command" module whose activity is both necessary and sufficient for aggressive action, a discovery that redefined long-held views of hypothalamic function More recently, her lab has explored how neuromodulators, including specific neuropeptides, dynamically tune these circuits to accommodate factors such as sex, internal state, and social context, providing fresh insights into the neural basis of violence, parenting, and territorial defense. Lin's contributions have been recognized by the Alfred P. Sloan Research Fellowship, McKnight Scholar Award, Klingenstein Fellowship, Janett Rosenberg Trubatch Career Development Award, Capranica Prize in Neuroethology, and the Irma T. Hirschl Career Scientist Award. A committed mentor and advocate for diversity in neuroscience, she has trained numerous postdoctoral fellows and graduate students who now lead laboratories around the world. At RegPep25 she will present "Neuropeptide Modulation of Social Conflict Circuits," highlighting cutting-edge findings on how peptide signals gate the transition from investigation to attack and offering therapeutic clues for disorders marked by pathological aggression.

Tuesday June 24, Afternoon

Keynote Symposium 1

Chair: Limei Zhang (IRPS President)

14:00 - 14:40 **Wilson Compton** (Deputy director of NIDA, NIH, USA)

Harnessing GLP-1 agonists to understand and treat addictions

14:40 - 15:20 **William Wisden** (Imperial College, London, UK)

The impact of molecular neurobiology on modern neuroendocrinology

15:20 - 16:00 **Luis de Lecea** (Stanford School of Medicine, USA)

Neuropeptides and control of behavioral states

KS1-1 Harnessing GLP-1 Agonists to Understand and Treat Addictions

Compton, Wilson

Deputy Director, Office of the Director, National Institute on Drug Abuse (NIDA), Bethesda, Maryland, USA

Glucagon-like peptide-1 (GLP-1) agonists have transformed the treatment of metabolic disorders, and their potential appears to extend beyond their traditional applications. Specifically, increasing evidence indicates that GLP-1 receptors modulate the brain's mesolimbic dopamine reward pathway, which plays a crucial role in drug reward and addiction. This keynote will discuss the preclinical and clinical evidence of GLP-1 agonists as potential therapies to combat substance use disorders.

Preclinical research has documented that GLP-1 signaling attenuates the reinforcing effects of addictive substances such as alcohol, nicotine, and opioids, including a blunting of their acute enhancing dopaminergic effects. Animal models of addiction have also shown that GLP-1 drugs can prevent relapse and reduce drug taking. Studies in humans based on electronic health records or clinical trials have also given evidence that GLP-1 agonist drugs reduce drug taking and improve long-term outcomes for various substance use disorders. Moreover, very recently, a randomized clinical trial reported that semaglutide, a GLP-1 agonist drug, reduced cravings and heavy alcohol drinking in patients with alcohol use disorder while also reducing cigarettes smoked per day in those who were also regular smokers. Finally, the anti-inflammatory effects of GLP-1 drugs might also be useful in counteracting addiction-related changes in energy homeostasis and stress reactivity, giving a holistic framework for understanding addiction as a disorder of integrated systems.

Leveraging GLP-1-based therapies for addiction treatment presents a unique translational opportunity for bringing into the clinic the first class of medications to treat transdiagnostic substance use disorders but it also highlights the need to ensure that its development proceeds in a way that its accessible to all who need it.

The author declares no competing interests.

KS1-2 The impact of molecular neurobiology on modern neuroendocrinology

Wisden, William

Department of Life Sciences, Imperial College, London, UK

I will trace the "creation story" of neuroendocrinology and explore how molecular biology has shaped its development, highlighting why understanding this history is crucial as we reflect on the 50th anniversary of the IRPS. What we commonly recognize as "molecular biology" is centered around DNA engineering, which began with the discovery of restriction enzymes. Molecular biology acted as a radical catalyst, allowing cDNA cloning and sequencing, and the development of transgenic knock-in and knockout mice. Endocrinology was further accelerated by PCR-based bio-building, driving the molecularization of viral vector construction, and the development of DNA-encoded light- and chemo-

sensors to study brain circuits. It's interesting to note that in 1977, the Nobel Prize was awarded to Guillemin and Schally for their work with "old" protein molecular biology, and their discoveries concerning "the peptide hormone production of the brain". Just a year later, Nakanishi & Numa reported the sequencing of POMC's cDNA using the emerging "new" nucxiaoc acid molecular biology. This shift in approach was of equal significance to the understanding of peptides like growth hormone, insulin LH-RH, TRH, and somatostatin.

The author declares no competing interests.

KS1-3 Neuropeptides and control of behavioral states

de Lecea, Luis

Stanford University School of Medicine

Neuropeptides modulate the activity of neuronal circuits at multiple timescales, in conjunction with fast neurotransmitters. Vigilance states are highly regulated by neuropeptides: deficiency of the hypocretin (also known as orexin) peptides leads to narcolepsy, a sleep disorder characterized by sleep instability and intrusions of REM sleep into wakefulness. During the past twenty years, our laboratory has identified neuronal circuits associated with sleep/wake stability across the lifespan. We recently showed that sleep fragmentation associated with aging is caused by hyperexcitability of Hcrt neurons driven by reduced activity of Voltage dependent potassium channels KCNQ2/3. We have also identified new peptidergic circuits in the brainstem that predict the onset and duration of REM sleep episodes. These data highlight the prominent role of slow peptidergic transmission in driving brain state transitions.

The author declares no competing interests.

Tuesday June 24, Afternoon

Workshop on translational aspects of peptide GPCRs

Chair: Bob Millar

16:10 - 16:35 **WS1: Bob Millar** (University of Pretoria, South Africa)
Rescue of mutant peptide GPCRs in the HPG axis with small molecules: a more viable therapy than gene editing

16:35 - 17:00 **WS2: Terry Moody** (NIH, USA)
Leveraging receptor visualization to cancer treatment: bombesin and theragnostics

17:00 - 17:25 **WS1: Mary Lee** (VA, Washington DC, USA)
GLP-1R agonists and sodium glucose transport inhibitors in treatment of dementia

17:25 - 17:30 General discussion

WS-1 Rescue of function in human mutant peptide GPCRs with cell permeant small molecules: a more viable approach than gene therapy

Millar, Robert P., 1; Newton, Claire L., 1; Anderson, Ross C., 1;

1 Centre for Neuroendocrinology, Departments of Immunology and Physiology. University of Pretoria, Pretoria, South Africa; 2 Institute for Infectious Diseases, University of Cape Town, Cape Town, South Africa

G-protein coupled receptors (GPCRs) convey 80% of signalling across cell membranes and hence inactivating mutations give rise to diverse pathologies.
Reproduction in vertebrates is driven by hypothalamic peptides, kisspeptin and neurokinin B which stimulate gonadotropin releasing hormone (GnRH), which in turn stimulates luteinizing hormone (LH) and follicle stimulating hormone (FSH) which regulate testis and ovarian function.
Inactivating mutations in G-protein coupled receptors (GPCRs) at all levels of this axis give rise to incomplete reproductive development and adult infertility. The majority of the mutations in these GPCRs cause misfolding of the receptor and a failure to traffic to the cell surface. We have therefore sought for cell permeant small molecules which can bind orthosterically or allosterically to stabilize the nascent GPCR in the endoplasmic reticulum and chaperone the mutant GPCR to the cell membrane.
We have successfully identified cell-permeant small molecules targeting receptors at all levels of the axis and demonstrated rescue of cell surface expression and restoration of function for NKB, GnRH, LH and FSH receptors. Moreover we have also been able to allosterically activate binding deficient and signalling deficient LHR with an LHR small molecule.
These discoveries represent an advance towards personalized medicine for GPCR inactivating mutations in the human reproductive hormone axis. As GPCRs constitute 80% of signalling in humans, inactivating mutations are likely to be a major contributor of disease and hence targets for small molecule rescue of function. The existence of vast numbers of GPCR-targeted small molecules from pharma data bases, many of which have already entered the clinic but abandoned, provides a rich source for treating GPCR human mutations and is more viable than a gene repair approach.

The authors declare no competing interests.

WS-2 Leveraging receptor visualization to cancer treatment: Bombesin and theranostics

Moody, Terry W., 1; Jensen, Robert T., 2

1 NCI, CCT, Bethesda, Maryland, USA; 2 NIDDK, DDB, Bethesda, Maryland, USA

Bombesin (BB) is a 14 amino acid which is biologically active in the CNS and cancer. A type 1 GPCR (BB2R) binds BB and gastrin releasing peptide (GRP) but not neuromedin B (NMB), with high affinity. BB2Rs interact with Gq and are present on presynaptic neurons in the dorsal horn of the rat spinal cord which are associated with pruritus (Pagani et et al., Neuron 2019). High levels of GRP are present in the rat hypothalamus, where it causes satiety, and human small cell lung cancer (SCLC) where it stimulates growth. BB2Rs are present in the neuroendocrine SCLC and epithelial non-SCLC cells. NSCLC cells have ErbB receptor tyrosine kinases (RTKs) including the EGFR, HER2, HER3 and HER4. Adding GRP to NSCLC cells increases the phosphorylation of tyrosine amino acids (PY) of the ErbB RTKs by transactivation. Adding GRP to NSCLC cells increases PY1068-EGFR and P-ERK which is impaired by PD176252 (BB2R antagonist), gefitinib (RTK inhibitor), PP2 (Src inhibitor) and diphenyleneiodonium (NOX and DUOX inhibitor which reduces reactive oxygen species). The growth of NSCLC cells and tumors is stimulated by EGF or BB but inhibited by gefitinib or PD176252. The results indicate that transactivation of ERbBs is important in regulating NSCLC proliferation as well as well the

growth of CNS tumors. In glioblastoma (GBM) patients, the EGFR is often amplified or mutated (An et al., Oncogene, 2018). The growth of GBM cells and tumors is inhibited by BB2R antagonists or the chemotherapeutic temozolomide (De Oliver et al., 2009). The overexpression of BB2Rs by tumor cells is increasingly being used to both image and treat various neoplasms. 68Ga-NOTA-ACA-BB (BB2R agonist) is used to image GBM tumors in patients using PET techniques (Zhang et al, 2019). 177Lu-DOTA-labeled BB2R antagonists/agonists are increasingly being used for peptide receptor radioligand therapy (PRRT) to treat patients with breast, prostate, pancreatic or CNS tumors (Nock et al., 2023). The antagonist SAR-BB serves as a theranostic agent in that it can localize breast cancer tumors when labelled with 68Ga and used for treatment when labeled with 177Lu (Al-ibraheem et al., Sem Nucl Med, 2024). The treatment of neuroendocrine tumors with the somatostatin receptor agonist 177Lu-DOTATATE is highly effective and safe resulting in its approval by the FDA (DiFranco et al., 2024). It remains to be determined if 177Lu-BB2R ligands will be useful in treating cancer patients who do not respond to somatostatin analogs.

Supported by the NIH intramural program.

WS-3 GLP-1R agonists and sodium glucose transport inhibitors in treatment of dementia

Lee, Mary R., 1; Sen, Sabyascahi, 1; Ahmed, A., 1; Zen, Qing T., 1
1 Veterans Affairs Medical Center, Washington DC, USA

Background: Type 2 diabetes mellitus (T2DM) is a risk factor for Alzheimer's Disease and Alzheimer's Disease Related Disorders (AD/ADRD). Findings from observational studies suggest that glucagon-like peptide-1 receptor agonist (GLP-1RA) medications may lower the risk of AD/ADRD in individuals with T2DM. However, less is known about this effect in comparison to the most prescribed T2DM treatment, metformin and/or insulin, or in comparison to an active comparator such as sodium-glucose cotransporter 2 inhibitor (SGLT2i) medications which exert comparable glycemic control while acting via distinct mechanisms to lower glucose.

Methods: From the VA national electronic health record (EHR), we identified 1,165,436 Veterans with T2DM free of baseline AD/ADRD. Of them, 44,631 were initiated on GLP1a, 52,345 were initiated on SGLT2i, and 1,120805 were treated with on metformin and or insulin. Using propensity score for GLP1a initiation we assembled two matched cohorts, while remaining blinded to outcomes: a cohort of 33,133 pairs of patients receiving GLP1a vs. metformin-insulin and a cohort, 36,548 pairs of patients receiving GLP1a vs. SGLT2i. Cause-specific hazard ratio (95% CI) for the risk of ADRD during 7 and 4 years of follow-up, respectively, was estimated accounting for the competing risk of death.

Results: Incident AD/ADRD occurred in 1.8% (599/33,133) and 3.6% (1187/33,133) of the patients in the GLP1a and metformin-insulin groups, respectively. When compared with patients initiated on metformin and/or insulin, those who were also initiated on GLP-1RA medications had a significantly 28% lower risk (95% CI, 20% to 35%)] of developing new-onset AD/ADRD. There was no significant difference in risk for incident AD/ADRD between GLP1a and SGLT2i groups.

Conclusion: These findings suggest that either GLP-1RA or SGLT2i use may be associated with a lower risk of AD/ADRD in patients with T2DM. Future prospective studies need to examine this association in randomized controlled trials in patients with and without T2DM.

Supported by VA Medical Center, Washington DC.

Scientific Session 5

SS5: Peptides Involved in Energy Regulation

Chair: Jean-Louis Charli (Mexico)

08:00 - 08:05 Introduction

08:05 - 08:30 **Jean-Louis Charli** (UNAM, Mexico)

The TRH degrading ectopeptidase and energy homeostasis

08:30 - 08:55 **Patricia Joseph-Bravo** (UNAM, Mexico)

Hypophysiotropic TRH neurons and the thyroid axis are regulated by energy demands in a sex dependent manner

08:55 - 09:20 **Margarita Curras-Collazo** UC Riverside, USA)

CCK-AR-expressing Vagal Sensory Afferents of the Gut-Brain Axis Contribute to Pro-inflammatory IL-6 Response to LPS

09:20 - 09:45 **Lucila K. Elias** (Uni. Sao Paolo, Ribeiro Preto, Brazil)

Neuroendocrine regulation of energy homeostasis

09:45 - 09:50 General discussion/conclusion

SS5-1 The TRH-degrading ectopeptidase and energy homeostasis

Charli, Jean-Louis
Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico

In contrast to most peptidases that control the extracellular turnover of peptides, the specificity of thyrotropin-releasing hormone-degrading ectopeptidase (TRH-DE) is narrow, TRH being its only known biological substrate. TRH is a peptide produced by hypophysiotropic neurons of the paraventricular nucleus of the hypothalamus. As for TRH, the major sites of expression of TRH-DE are in the brain, including the tanycytes that line the ventral part of the third ventricle. In the outer layer of ME, TRH-DE is expressed on the surface of the end feet of β2-tanycytes, proximal to the TRH terminals and the portal vessels that connect to the anterior pituitary, where the peptide stimulates the secretion of thyrotropin (TSH). This anatomical arrangement suggests that TRH-DE might hydrolyse TRH after its release from TRH terminals, before it reaches the pituitary and thus control thyroid axis activity. However, in rodents fed a normocaloric diet, ip injection of specific inhibitors did not change the basal concentration of TSH in rat serum. and the phenotype of mice KO for Trhde was not substantially different from that of wild type mice. Furthermore, the manipulation of TRH-DE expression in ME with AAV1 vectors barely changed the central or peripheral parameters of the activity of the rat thyroid axis. Nevertheless, if rodents were challenged by altering their energy balance, we observed dramatic consequences on thyroid axis activity and metabolism. In rats, ip injection of specific inhibitors of TRH-DE amplified, while overexpression of TRH-DE in ME attenuated the effect of cold stress on serum TSH concentration. Under a high-caloric diet, the phenotype of Trhde KO mice consisted of increased energy expenditure and improvement of glycemia, body weight and composition. Finally, the responses of the thyroid axis, as well as of carbohydrate and lipid metabolism, to fasting were altered in Trhde KO mice, suggesting an inefficient shut down of the thyroid axis. In conclusion, we established that TRH-DE is necessary to adjust metabolism when rodents are challenged by changes in energy balance. We postulate that it is the β2-tanycyte that is the critical locus for the control of metabolism by TRH-DE, although the role of other central sites of expression should be investigated due to the anorexic properties of TRH.
Supported by DGAPA-UNAM (PAPITT IN216022 and IN220825) and CONAHCYT (CBF2023-2024-732).

SS5-2 Hypophysiotropic TRH neurons and the thyroid axis are regulated by energy demands in a sex dependent manner

Joseph-Bravo, P.
Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico

Thyrotropin-releasing hormone (TRH) from the hypothalamic paraventricular nucleus (PVN) triggers the activity of the hypothalamus-pituitary-thyroid (HPT) axis. Thyroid hormones regulate thermogenesis and basal metabolic rate, carbohydrate and lipid metabolism, and mobilize energy substrates to oxidizing tissues. Energy demands activate, whereas energy deficits inhibit PVN Trh expression and HPT axis activity in male rodents. Cold exposure or voluntary exercise enhance the expression of Trh in the PVN

of male rats, but these effects are curtailed by previous stress perceived as adults or during neonatal development; stress prevents also the adequate response of brown adipose tissue (BAT) or white fat (WAT) loss. Trh expression is not increased in females and responses also differ after fasting; Trh expression decreases faster in adolescent females than males (at 24h) while the opposite occurs in adults (48h). We recently characterized the cold-response of female HPT axis; Trh expression increases later than in males, so as HPT and BAT activities. Although Trh expression was not enhanced in females after voluntary exercise, serum concentration of TSH and slightly that of T4 were, supporting activation of HPT axis, prevented by previous chronic stress that reduced distance and prevented fat loss. As exercise (Ex) decreases food intake voluntarily, results were compared to pair-fed groups. Because changes observed after stress/Ex on HPT axis parameters were subtle compared to those after cold or fasting, we evaluated gene expression of T3-targets in tissues involved in exercise. Chronic variable stress (CVS) was applied to adolescent male and female rats, exposed as adults to voluntary exercise and compared to naïve or to pair-fed sedentary rats. The pair-fed rats had the reported changes of mild calorie restriction compared to adlib sedentary controls, exercise counteracted all changes except for higher expression of Pomc and Dio2, Trh expression remained low in females while increased in males, serum concentration of leptin remained low, and corticosterone lower. CVS affected more females than males, exacerbated many effects of pair-feeding and blunted those of exercise in both sexes. The expression profile of differentially regulated genes in skeletal muscle (SKM), subcutaneous and visceral WATs and in BAT showed many similarities between sexes: exercise increased expression of Adrb3 in adipose tissues, that of Dio2 in adipose tissues and SKM, Pgc1a in SKM and Pparg in WAT; CVS repressed these increases in all except in female BAT. These similar patterns support the proposed concerted response and crosstalk communication between these tissues. Main sex differences induced by CVS were higher circulating levels of FT3 in females than in males, which could be due to the high Dio2 expression in BAT, not inhibited as in all other tissues. Since BAT Ucp1 expression was still inhibited in CVS-Ex rats, the physiological consequences of high FT3 serum concentration remain to be explained and whether it is a plausible mechanism of the high FT3 levels detected in humans with PTSD. Stressed-induced HPT disfunction may contribute to the symptoms of subclinical hypothyroidism that are more frequent in women.

Supported by DGAPA-UNAM IN217422, IN227825.

SS5-3 CCKA receptor containing vagal sensory afferents of the gut-brain axis contribute to the pro-inflammatory interleukin-6 response to lipopolysaccharide treatment

Curras-Collazo, Margarita
University of California, Riverside, California, USA

Gulf War Illness (GWI) is a chronic condition marked by unexplained neurological and gastrointestinal symptoms affecting Gulf War Veterans. They experience cognitive deficits, chronic fatigue and neuroinflammation and gut dysbiosis. It is therefore suspected that altered gut-brain signaling plays a prominent role in GWI. Vagal afferent neurons (VANs) are emerging as important mediators of the gut-brain axis, making them potential targets for understanding GWI pathophysiology. To examine whether

cholecystokinin (CCK) A receptor-containing VANs contribute to gut-brain immune signaling, adult male mice were subjected to bilateral vagal deafferentation via injection of CCK conjugated with the neurotoxin saporin (CCK-SAP, 250nL, 250ng/uL), into the nodose ganglia which contains VANs that project to solitary tract nucleus (NTS). Controls received injections with Blank-SAP. Following 6d of deafferentation, mice were injected with 0.1mg/kg LPS and plasma, brains, and nodose ganglia were collected 2h later. LPS increased plasma levels of the pro-inflammatory cytokine IL-6 to a lesser extent in CCK-SAP (66%) than BLANK-SAP (123.2%). cFOS-positive cell counts in the intermediate NTS were 31% lower in CCK-SAP than in BLANK-SAP ($p<0.05$, n=4-6/group), indicating deafferentation by CCK-SAP. Another cohort of CCK-SAP mice showed no effect of treatment with CCK, a satiety peptide, on food consumption as seen at baseline prior to surgery ($p<0.01$-0.0001, n=7). Treatment with CCK, which is insulinotropic, reduced the peak response at 15 and 30 min post-glucose injection ($p<0.01$-$.05$, n=4). Therefore, the glucose tolerance response may also be used to gauge CCK-SAP method. Collectively, the findings indicate that CCKAR+ VANs, suggesting that CCKAR-containing VANS participate in pro-inflammatory responses and can be leveraged to interrogate gut-brain immune functioning in GWI.
Supported by DoD grant (MCC), UCR Student Minigrants (SA, JS)

SS5-4 **Neuroendocrine regulation of energy homeostasis**

de Jesus, Aline, 1; Gonçalves, Gabriel, 1; Santos, Raoni, 1; Barbosa, Patrik, 1; Leao, Ricardo, 1; Antunes-Rodrigues, Jose, 1; Elias, Lucila 1
1 Department of Physiology, Ribeirao Preto Medical School, University of Sao Paulo, Sao Paulo, Brazil

The high prevalence of obesity has pursued the expansion of investigation on brain circuitries and molecular mechanisms involved in the neuroendocrine control of energy homeostasis. Hypothalamus plays a crucial role in the regulation of appetite, satiety responses and also energy expenditures. STAT3 and PI3K are known to mediate leptin effects on central regulation of energy homeostasis. We have investigated the role of hypothalamic neurons on food intake and energy expenditure using conditional deletion through cre-lox method, chemogenetic and specific brain site stimulation with different drugs or hormones. We have demonstrated that specific deletion of STAT3 in SF-1 neurons in the ventromedial hypothalamic (VMH) neurons increases body weight and food intake under a high fat diet, but not in animals treated with a regular diet. Sex difference was observed, since female mice were more affected by this STAT3 deletion, showing also a decrease in the energy expenditure. Deletion of P110α in SF1 neurons also increased body weight, but not food intake, and it decreased energy expenditure and brown adipose tissue thermogenesis in high fat diet treated animals. We also demonstrated that P110α signaling in SF1 neurons is required for the effects of estradiol on energy homeostasis. These data demonstrate that STAT3 e PI3K signaling in VMH neurons are required for the regulation of energy homeostasis to protect against obesogenic diet.
Supported by São Paulo Research Foundation - FAPESP, National Council for Scientific and Technological Development - CNPq.

Scientific Session 6

SS6: Neuropeptidergic Integration of Emotion, Fear, and Social Behavior

Chair: Joanna Dabrowska (USA)

08:00 - 08:05 Introduction

08:05 - 08:30 **Pablo Castillo (**Albert Einstein College of Medicine, USA)

Presynaptic BDNF/TrkB Signaling Drives Axonal Local Translation Essential for Long-Term Plasticity

08:30 - 08:55 **Eric G. Krause** (Georgia State University, USA)

The integration of interoceptive signals and behavioral responses via oxytocin receptors

08:55 - 09:20 **Zhihua Gao** (Zhejiang University, China)

The role of oxytocin in emotional control

09:20 - 09:45 **Joanna Dabrowska** (Rosalind Franklin Univ., USA)

Vasopressin and oxytocin integrate interoceptive signals and fear memory in extended amygdala

09:45 - 09:50 General discussion/conclusion

(SS6-1 Oxytocin activity in the Paraventricular and Supramammillary Nuclei of the Hypothalamus is crucial for social recognition memory in rats) (Talk canceled)

Rajamani, Keerthi Thirtamara, 1, 2; Barbier, Marie, 1; Lefevre, Arthur, 3, 4; Niblo, Kristi, 1; Cordero, Nicholas, 5; Netser, Shai, 6; Grinevich, Valery, 3; Wagner, Shlomo, 6; Harony-Nicolas, Hala 1, 7, 8, 9

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Social recognition memory (SRM) is essential for social interactions and is conserved across species, including rodents. It enables individuals to acquire, retain, and recall conspecific identities, facilitating social organization. Impairments in SRM are observed in psychiatric disorders such as autism spectrum disorder and schizophrenia, highlighting the need to identify its underlying neural mechanisms.

Oxytocin (OXT), synthesized in the paraventricular (PVH), supraoptic (SON), and accessory hypothalamic nuclei, plays a key role in social behaviors, including maternal care, bonding, and SRM. However, the specific hypothalamic nuclei required for SRM formation and their downstream targets remain unclear. We hypothesized that PVH-OXT neurons are essential for both short- and long-term SRM. Using DREADDs (designer receptors exclusively activated by designer drugs) to selectively inhibit OXT neurons in the PVH of Sprague Dawley rats, we found significant impairments in both short- and long-term SRM.

To identify downstream targets involved in SRM, we examined the supramammillary nucleus (SuM), known for its role in hippocampal-dependent learning and memory. Immunohistochemistry with OXT-specific antibodies confirmed the presence of OXT fibers in the SuM. Anterograde tracing (AAV-OXTp-Venus and OXTp-Synaptophysin-GFP) revealed that these fibers originate from the PVH but not the SON. In situ fluorescent hybridization further demonstrated that OXT receptors in the SuM are primarily expressed on glutamatergic neurons, including those projecting to the CA2 region of the hippocampus. Finally, pharmacological blockade of OXT receptors in the SuM disrupted both short- and long-term SRM.

Taken together, our study discovered a previously undescribed role for the SuM in regulating SRM via OXT signaling and reinforces the specific role of PVH-OXT neurons in regulating this form of memory.

The authors declare no competing interests.

SS6-1 Presynaptic BDNF/TrkB Signaling Drives Axonal Local Translation Essential for Long-Term Plasticity

Ernesto Griego[1,5], Shivani C. Kharod[1,5], Joongkyu Park[2], Robert H. Singer[3], Young J. Yoon[1,3] and Pablo E. Castillo[1,4]

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Local synaptic protein synthesis endows remote neuronal compartments with the ability to rapidly respond and adapt to local cues. Growing evidence indicates that some forms of presynaptic long-term plasticity in the mature mammalian brain rely on axonal protein synthesis, but how this process is regulated remains poorly understood. Brain-derived neurotrophic factor (BDNF) and its cognate receptor TrkB promote local protein synthesis in dendrites and play a key role in postsynaptic plasticity. Much less is known about the role of BDNF/TrkB and local translation in presynaptic plasticity. We hypothesize that BDNF/TrkB signaling triggers presynaptic local protein synthesis. To test this hypothesis, we examined the hippocampal mossy fiber to CA3 pyramidal cellsynapse (MF-CA3), which expresses both structural and functional presynaptic plasticity. Importantly, MFs also express uniquely high levels of BDNF, which is released upon MF repetitive stimulation. We found that inhibiting presynaptic protein synthesis by expressing a genetically encoded inhibitor targeted to the presynaptic compartment blocked MF-LTP but not short-term plasticity. Moreover, presynaptic *Bdnf* or *Trkb*conditional deletion from granule cells (*Bdnf* and *TrkB* cKO), significantly reduced MF-LTP and LTP-induced presynaptic translation. In contrast, basal synaptic transmission and short-term plasticity remained unchanged. Remarkably, BDNF replenishment following MF repetitive stimulation required local BDNF synthesis. Altogether, our findings demonstrate that BDNF is locally synthesized in MF boutons and that autocrine BDNF/TrkB signaling plays a pivotal role in regulating local translation, which is essential for long-term plasticity.
Supported by R01 MH125772, R01 NS113600, and R01 MH 116673 to PEC, and R35 GM136296 to RHS.

SS6-2 The integration of interoceptive signals and behavioral responses via oxytocin receptors
Krause, Eric
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Interoception broadly refers to awareness of one's internal milieu. Vagal sensory afferents monitor the internal milieu and maintain homeostasis by engaging brain circuits that alter physiology and behavior. While the importance of the body-to-brain communication that underlies interoception is implicit, the vagal afferents and corresponding brain circuits that shape perception of the viscera are largely unknown. Here, we use mice to parse neural circuits subserving interoception of the heart and gut. We determine vagal sensory afferents expressing the oxytocin receptor, hereafter referred to as NDGOxtr, send projections to the aortic arch or stomach and duodenum with molecular and structural features indicative of mechanosensation. Chemogenetic excitation of NDGOxtr significantly decreases food and water consumption, and remarkably, produces a torpor-like phenotype characterized by reductions in cardiac output, body temperature, and energy expenditure. Chemogenetic excitation of NDGOxtr also creates patterns of brain activity associated with augmented hypothalamic-pituitary-adrenal axis activity and behavioral indices of vigilance. Recurrent excitation of NDGOxtr suppresses food intake and lowers body mass, indicating that mechanosensation of the heart and gut can exert enduring effects on energy balance. These findings suggest that the sensation of vascular stretch and gastrointestinal distention may have profound effects on whole body metabolism and mental health.
The author declares no competing interests.

SS6-3 The oxytocinergic and behavioral signatures of lactation
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Understanding lactation is important for reproductive health. While pulsatile release is known to drive milk ejection, how oxytocin neurons coordinate lactation and how it manifests in behavior remains poorly defined. Here,

we combine in vivo calcium recording, intramammary pressure (IMP) monitoring and behavioral tracking to investigate the neural-behavioral interplay of lactation in conscious rats. We show that hypothalamic oxytocin neurons exhibit synchronized, episodic firing during nursing, which remains stable throughout lactation but attenuates under anesthesia. Such oxytocinergic firing drives IMP surge and stereotypic behavioral interactions in both dam and pups. Blocking oxytocin receptors attenuates milk ejections and behavioral actions, suggesting behavioral correlation to milk ejections. Leveraging the behavioral features, we construct a deep-learning framework to automatically identify milk ejections from videos, preventing intensive labor and invasive procedures. Our study uncovers the oxytocinergic and behavioral signatures of lactation and provides a scalable approach for further investigation.

*The authors declare no competing interests.*SS6-4 **The role of vasopressin and oxytocin receptors in the integration of interoceptive states and fear memory formation in the extended amygdala**

Francesconi, Walter, 1; Olivera-Pasilio, Valentina, 1; Berton, Fulvia, 1; Olson, Susan L., 1; Chudoba, Rachel, 1; Monroy Lorena, M., 1; Krabichler, Quirin, 2; Grinevich, Valery, 2; Dabrowska, Joanna 1

1 Center for Neurobiology of Stress Resilience and Psychiatric Disorders, The Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, Illinois, USA; 2 Department of Neuropeptide Research in Psychiatry, German Center for Mental Health (DZPG), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Internal physiological signals dynamically interact with the environment to shape appropriate defensive behaviors. Neurons in the hypothalamus regulate internal states, such as thirst/electrolyte balance and circadian rhythmicity, and send projections to the bed nucleus of the stria terminalis (BNST) to influence defensive responses, including fear and anxiety-like behaviors. Hypothalamic hormone and neuromodulator, arginine-vasopressin (AVP) modulates water and electrolyte balance and is involved in circadian rhythmicity. The dorsolateral BNST (BNSTDL) expresses oxytocin (OTR) and vasopressin receptors but their roles in regulating BNSTDL activity is not known. Here, we show that AVP directly excites BNSTDL neurons, which requires OTR but not vasopressin 1a (V1aR) or 1b (V1bR) receptors. In addition, we show that selective V1aR agonist demonstrates a moderate excitatory effect in the subset of BNSTDL neurons. These excitatory effects of AVP are confirmed by specifically recording excitability of fluorescent OTR-neurons from OTR-Cre transgenic male rats after application of AVP, oxytocin (OT) or selective OTR agonist, TGOT. Considering the well-established role of BNSTDL in avoidance and fear-related behaviors, we next used chemogenetics in OTR-Cre rats and demonstrate that silencing of OTR-BNSTDL neurons significantly reduces exploration of open arms of the elevated plus-maze and increases anxious arousal in the fear-potentiated startle. Lastly, using AVP-Cre rats we show that the BNSTDL receives functional AVP innervation from the supraoptic and suprachiasmatic nuclei of the hypothalamus. Overall, we demonstrate how OTR-BNSTDL neurons exited by hypothalamic AVP play a major role in regulating BNSTDL excitability, overcoming threat avoidance, and reducing fear responses to ambiguous threats. Therefore, changes in the activity of internal state-sensitive hypothalamic nuclei will directly impact OTR neuron excitability in the BNSTDL via hypothalamic inputs to shape appropriate, physiologically relevant levels of defensive behaviors.

Supported by grant MH113007 from the National Institutes of Health (NIH), National Institute of Mental Health (NIMH) to JD and by the Synergy European Research Council (ERC) grant "OxytocINspace" 101071777, SFB Consortium 1158-3, and German-Israeli Project cooperation (DIP) GR3619-1, and ERANET-Neuron GR 3619/25-1 to VG.

Scientific Session 7

SS7: Neuropeptides and Hormonal Networks in Systemic Physiological Regulation

Chair: Teresa Morales (UNAM, Mexico)

10:00 - 10:05	Introduction
10:05 - 10:30	**Eric Lazartigues** (Louisiana, USA) ***miRNA targeting for the renin-angiotensin system***
10:30 - 10:55	**Paul Marvar** (Washington DC, USA) ***The Brain Angiotensin System: Connecting Cognition, Stress, and Cardiovascular Disease***
10:55 - 11:20	**Lei Xiao** (Fudan University, China) ***Hypothalamic Oxytocinergic System-Neurons, circuits and functions***
11:20 - 11:45	**Teresa Morales** (Mexico) ***Prolactin Dysregulation in a Mice Model of Kidney Disease***
11:45 - 11:50	General discussion/conclusion

SS7-1 **miRNA targeting for the renin-angiotensin system**

Tarhriz, Vahideh, 1, 2; Xia, Huijing, 1, 2; Patel, Ayushi, 1; and Lazartigues, Eric, 1, 2

1 Cardiovascular Center of Excellence, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA; 2 Southeast Louisiana Veterans Health Care System, New Orleans, Louisiana, USA

Hypertension is a complex, multifactorial disease influenced by sex hormones, with sex differences playing a significant role in its development and progression. While non-coding RNAs, particularly microRNAs (miRNAs), have been implicated in hypertension regulation, their precise roles remain unclear. This study aims to investigate sex-specific miRNA expression in a salt-sensitive hypertensive model, providing insights into potential sex-dependent regulatory mechanisms.
Male and female C57BL/6J mice were subjected to DOCA-salt-induced hypertension. Extracellular vesicles (EVs) were isolated from the cerebral spinal fluid and plasma and analyzed for miRNA expression profiles using next-generation sequencing. Hypothalamic tissue and plasma samples were collected from control, DOCA-salt, ovariectomized-DOCA-salt, and orchiectomized-DOCA-salt groups to evaluate miRNAs identified through in silico analysis and their association with sex-specific hypertension mechanisms.
Our findings revealed significant sex-specific miRNA expression differences in both plasma and hypothalamic samples. For example, miR-206-3p was upregulated in male DOCA-salt mice, while miR-125-5p was elevated in both sexes; however, expression of the latter was reduced in OCX-DOCA-salt and unchanged in OVX-DOCA-salt mice rather than the DOCA-Salt mice. Additionally, miR-486-5p showed opposing trends between sexes, suggesting a potential role in different sexes.
This study reveals the important role of sex-specific miRNAs in salt sensitive hypertension regulation, offering new opportunities for personalized, sex-dependent therapeutic strategies. The identification of miR-206-3p, miR-125-5p, and miR-486-5p as key regulatory miRNAs in hypertension highlights the hormonal influences on their expression and reinforces their functional significance in hypertension progression and treatment.

EL was supported in part by grants from the National Heart Lung and Blood Institute (150592 and HL163588) and the Veterans Administration (BX006387).

SS7-2 **The brain-angiotensin system: connecting cognition, stress and cardiovascular disease**

Marvar, Paul

Departments of Pharmacology & Physiology and Psychiatry & Behavioral Health The George Washington University, Washington, DC, USA

The brain renin-angiotensin system (bRAS) is a neuromodulatory neuroendocrine network traditionally known for regulating blood pressure, thirst, and stress responses. Despite decades of research, the mechanisms linking bRAS signaling, from peptides to receptors to neural circuit function, and its therapeutic potential in disorders such as PTSD and Alzheimer's disease remain incompletely understood.

This talk will present updated insights into bRAS function, from precursor (angiotensinogen) to peptide to receptor, with a focus on key subtypes (AT1R, AT2R, MasR), enzymatic regulators (ACE, ACE2), and the emerging role of astrocyte-derived angiotensinogen (Agt) in neural control of cardio-behavioral-stress regulation. We will highlight recent methodological advances using bRAS Cre-lox mouse models, LC/MS-driven angiotensin peptide profiling via enhanced capillary electrophoresis, and transcriptomic analyses from genetically diverse AD-BXD mice, which reveal age- and sex-specific alterations in bRAS-related genes in Alzheimer's-relevant brain regions. Overall, this work aims to reveal new molecular dimensions of bRAS function, offering deeper neurobiological insights and advancing its therapeutic potential at the intersection of stress-related cardiovascular and neurodegenerative disorders.

Supported by 1R21AG086859-01, CDMRPPR210574, and U24_AG072701 Pilot Award

SS7-3 **Diversity and complexity of the hypothalamic oxytocinergic system in neurons, circuits and functions**

Xiao, Lei

The State Key Laboratory of Medical Neurobiology, MOE Frontiers Center for Brain Science, and the Institutes of Brain Science, Fudan University, Shanghai, China

Endogenous oxytocin is predominantly synthesized by hypothalamic neurons. Despite only ~2000, ~8000, and ~50000 oxytocinergic neurons in mouse, rat, and human brains, hypothalamic oxytocinergic neurons orchestrate multimodal brain functions, spanning prosocial behavior, energy homeostasis, emotional regulation, and sensory processing through region-specific neuromodulatory pathways. Systematic interrogation of the hypothalamic oxytocinergic system through multiscale integration of cellular heterogeneity, circuit topographies, and neuromodulatory dynamics will elucidate its multifaceted roles in central nervous system (CNS) function while bridging mechanistic insights to targeted neurotherapeutic development. Our laboratory has conducted a series of integrative investigations targeting the oxytocinergic system, 1) dissecting the morpho-electric properties and diversity of PVN OXT neurons; 2) exploring the modulatory roles of oxytocinergic projections in different downstream regions; 3) delving the oxytocinergic alleviation functions and possible mechanisms for multiple brain diseases.

Supported by Noncommunicable Chronic Diseases-National Science and Technology Major Project (2023ZD0506800), National Natural Science Foundation of China (32371058, 81970727, 31900738), Shanghai Municipal Science and Technology Major Project (2018SHZDZX01), ZJ Lab, and Shanghai Center for Brain Science and Brain-Inspired Technology.

SS7-4 **Prolactin dysregulation in a mouse model of kidney disease**

Morales, Teresa, 1; Viñuela-Berni, Verónica, 1; Carbajo-Mata, María Antonieta, 2; Corona, Rebeca, 1

1 Laboratorio de Neuroanatomía Funcional y Neuroendocrinología, Instituto de Neurobiología UNAM, Campus Juriquilla, Querétaro, México; 2 Laboratorio Universitario de Bioterio, Instituto de Neurobiología UNAM, Campus Juriquilla, Querétaro, México

Kidney disease (KD) is a global health concern. Mexico has a high prevalence of KD, with diabetes and hypertension being the most common causes; these conditions are recognized as significant metabolic issues in the country. KD is characterized by both functional and structural abnormalities of the kidneys, and its classification depends on the cause, severity, and duration of these abnormalities. KD patients exhibit endocrine effects that, in turn, affect metabolic and systemic functions. Changes in circulating prolactin (PRL) levels are highly prevalent in KD patients. However, it remains unclear whether this accumulation results from impaired kidney filtration or altered mRNA synthesis at the pituitary level due to disrupted dopamine feedback. The present study generated a KD model in adult C57BL/6J female mice through intragastric adenine treatment (tx; 50 mg/kg, n=10) for four weeks to evaluate PRL levels. To track the progression of PRL disturbances in the KD, the female mice were divided into four groups based on the timing of sample collection. Mice were sacrificed at various intervals following adenine treatment to assess the changes throughout the progression of the disease, specifically: 1) after one day, 2) after three days, 3) after five days, 4) after one week, 5) after two weeks, 6) after three weeks, and 7) after the fourth week of treatment. Blood was collected from all groups. For group 7, sacrificed after four weeks of treatment, kidneys and urine were also collected, and body weight was recorded. The kidney damage was confirmed in the fourth week after adenine treatment through metabolic, biochemical, and morphological parameters. As expected, serum creatinine and urea levels were significantly increased in the adenine-treated group. Additionally, hematoxylin-eosin histological kidney sections were analyzed, and significant differences in tubular dilation were found, which confirmed renal damage. Blood levels of PRL were determined by ELISA in all cases. Contrary to expectations, peripheral levels of PRL significantly decreased from the fifth day of adenine treatment and continued to decline through the fourth week of the KD model. Further experiments are necessary to uncover the mechanisms behind PRL accumulation in a KD mouse model.

Supported by UNAM-DGAPA-PAPIIT IN220025, IN214822 and IN205423.

Scientific Session 8

SS8: Neuropeptides and the Architecture of Sleep: Circuits, Clocks, and Cognitive Health

Chair: Bill Wisden (Imperial, UK)

10:00 - 10:05 Introduction

10:05 - 10:30 **Ruud Buijs** (UNAM, Mexico)

Vasopressin as neurotransmitter of the biological clock signals the rest period in physiology and activity

10:30 - 10:55 **Kyoko Tossell** (Imperial College, London, UK)

Role of prefrontal cortex somatostatin neurons directing top-down control of sleep preparatory behavior and sleep

10:55 - 11:20 **Jason Rihel** (UCL, UK)

Zebrafish as a model system to study role of galanin in regulating sleep homeostasis

11:20 - 11:45 **Sara Calafate** (Portugal)

Early alterations in the MCH system link aberrant neuronal activity and sleep disturbances in a mouse model of AD

11:45 - 11:50 General discussion/conclusion

SS8-1 **Vasopressin, as a neurotransmitter of the biological clock, signals the rest period in physiology and activity**

Buijs, Ruud M., 1; Hurtado Alvarado, Gaby., 1; Santacruz Martinez, Esteban., 1; Romero Vera, Ilse, 1

1 Instituto Investigaciones Biomedicas, Fac. Medicina dept. Anat. Universidad Nacional autonoma de Mexico.

As one of the first discovered peptide hormones, vasopressin (VP) was also one of the first neuropeptides shown to act as a neurotransmitter. VP is also one of the transmitters of the Suprachiasmatic Nucleus (SCN), and the only peptide of the SCN that shows a precise rhythm in its secretion. The rhythm of clock genes in SCN neurons and the light/dark cycle drives VP production and release.For a long time, the group of Reppert et al. has demonstrated that its release increases just before the onset of the light period in nocturnal rodents.

Using microdialysis of VP or its antagonists, we have shown that VP inhibits corticosterone secretion in the area of the Paraventricular nucleus. In the medial preoptic area, it inhibits Gonadotrophin secretion. In the Arcuate nucleus, VP promotes glucose transport via tanycytes into the nucleus, thus lowering peripheral glucose levels at the beginning of the sleep phase. Currently, we study how VP is involved in inhibiting locomotor activity.

From hormonal levels to temperature and activity, all these circadian variables show large amplitudes over the 24-hour cycle whereby trough to peak value may change until a factor 10. However, despite these enormous 24-hour changes, the day-to-day variation in hormonal level or activity will be less than 5% at any particular hour at a specific time. Consequently, an elaborate feedback system must inform the SCN and other participating nuclei about the actual levels reached during the circadian cycle. Therefore, changes in activity or circulating levels in glucose or corticosterone are transmitted to SCN VP neurons, changing their neuronal activity and allowing for an adaptation in physiology or activity.

The authors declare no competing interests.

SS8-2 **Role of prefrontal cortex somatostatin neurons directing top-down control of sleep preparatory behaviour and sleep**

Tossell, Kyoko, 1; Franks, Nicholas P., 1,2 ; Wisden, William 1,2,3

1 Department of Life Sciences, Imperial College London, UK; 2 UK Dementia Research Institute, Imperial College London, UK; 3 Center of Neurotechnology, Imperial College London, UK

Animals undertake specific behaviours when sleep pressure increases, yet little is known about whether these innate behaviours, such as nest building, are controlled by intrinsic parts of the sleep-inducing circuitry. The prefrontal cortex (PFC) has an executive function and contributes to planning, and is particularly sensitive to sleep deprivation. We found that rare types of fast-spiking somatostatin-

expressing, GABAergic ($PFC^{Sst\text{-}GABA}$) neurons in mouse PFC become activated during sleep deprivation. These cells project to the lateral preoptic (LPO) and lateral hypothalamus (LH). Stimulating $PFC^{Sst\text{-}GABA}$ terminals in the LPO hypothalamus caused sleep-preparatory behaviour, while stimulating $PFC^{Sst\text{-}GABA}$ terminals in the LH mimicked recovery sleep in the absence of excessive fatigue. Furthermore, these $PFC^{Sst\text{-}GABA}$ terminals had enhanced activity during nesting and sleep, inducing inhibitory postsynaptic currents on diverse GABAergic cells in the respective sublocation of hypothalamus. Our findings provide a circuit link for how the PFC directly instructs the hypothalamus to ensure that optimal sleep takes place in a suitable place.

This work was supported by Wellcome Trust, UK (10839/Z/15/Z, N.P.F.;107841/Z/15/Z, W.W.; 220759/Z/20/Z, N.P.F. and W.W.), the UK Dementia Research Institute at Imperial College (W.W.and N.P.F.).

SS8-3 **Zebrafish as a model system to study the role of galanin in regulating sleep homeostasis**

Pittman, Talia, 1; Rihel, Jason, 1

1 Department of Cell and Developmental Biology, University College London, London, UK

Sleep pressure homeostatically increases during wake and dissipates during sleep, but the molecular signals and neuronal substrates that measure homeostatic sleep pressure remain poorly understood. In zebrafish we have previously demonstrated that preoptic Galanin-positive neurons are active during rebound sleep and that Galanin signalling is required for increased sleep in response to sleep pressure. Using Crispr-Cas9 mediated mutations of all four Galanin receptors in zebrafish, we are now mapping the downstream circuits required for mediating rebound sleep. We propose that galaninergic neurons integrate sleep pressure signals from global neuronal activity and act as an output arm for the vertebrate sleep homeostat via signalling on select downstream circuits expressing only a subset of galanin receptors. *The authors declare no competing interests.* \

SS8-4 **Sleep-dependent modulation of brain homeostasis in Alzheimer's Disease**

Seabra, F., 1; Ferreira, M., 1; Frei, J., 1; Oliveira, T., 1; Calafate, S., 1

1 Life and Health Science institute (ICVS)/ School of Medicine at University of Minho, Portugal

In Alzheimer's disease (AD), early accumulation of amyloid-β (Aβ) causes deficits in episodic memory, leading to cognitive impairment. Moreover, hippocampal hyperactivity and increased susceptibility for seizure emerges, placing synapse dysfunction is at the basis of AD pathogenesis. Remarkably, neuronal hyperactivity is prominent during sleep and sleep disturbances are accompanied by silent epileptic-like discharges in individuals with AD during the prodromal phase. Moreover, early sleep disturbance coincides with aberrant neuronal activity and neuroinflammation, in patients and mouse models.

Recently, using spatial transcriptomics and bulk RNAsequencing, we found that the sleep-active Melanin Concentrating Hormone (MCH) decreases the expression of immediate early genes and restrains synaptic activity of hippocampal neurons (Calafate et al, 2023). MCH-neurons located in the lateral hypothalamic area (LHA), regulate duration of rapid-eye movement (REM) sleep and project to the hippocampus modulating hippocampal-dependent memory.

Our work identified an early impairment of the MCH-system in AD patients and in the AppNL-G-F mice (a mouse model for early stages of AD) at the time point that aberrant hippocampal activity, impaired REM sleep and higher susceptibility for seizures emerge. Moreover, we showed that MCH peptide is sufficient to rescue increased hippocampal excitatory synaptic transmission in the AppNL-G-F mice.

However, brain homeostasis depends on complex interaction between cell-types. Currently, we are studying how the MCH-system modulates neuron-microglia interactions and how it impacts brain homeostasis.

The authors declare no competing interests

PL5: Plenary Lecture: Peptide control of energy balance

13:00 - 13:45 **Scott Kanoski** (USC, USA Viktor Mutt Lecturer)
introduced by **Terry Davidson (AU)**

Scott Kanoski, Diabetes & Obesity Research Institute, University of Southern California, Los Angeles, California, USA

Maintaining energy balance requires the regulation of processes that determine when, what, and how much food to consume. Various peptides, particularly those derived from the gastrointestinal tract, are known to regulate the processes of satiation (becoming full and terminating a meal) and satiety (post-meal state without appetite). Knowledge of peptide control of satiation and satiety has led to recent advances in diabetes and obesity pharmacotherapy, with glucagon-like peptide-1-based drugs currently leading the way. However, much less is known about the peptidergic control of the processes that promote energy intake, including appetite (leading to food seeking and meal initiation) and "appetition", which is a post-oral positive feedback process occurring early in a meal to promote further caloric consumption. This presentation reviews findings identifying the neural circuitry through which the stomach-derived hormone, ghrelin, increases appetitive processes, including food cue-potentiated eating. Additional results will be highlighted that identify a role for the hypothalamic neuropeptide, melanin-concentrating hormone, in mediating the post-oral process of appetition. A clearer understanding of peptidergic control of appetite and appetition may lead to novel advances in obesity treatment.

Supported by grants R01DK104897, R01DK123423, R01DK118402 from the National Institutes of Health.

About the speaker

Scott Kanoski is a Professor in Diabetes & Obesity Research Institute, University of Southern California. He has been awarded the esteemed **RegPep25 Viktor Mutt Lectureship**, a recognition reserved for scientists less than 50 years old, who have made transformative contributions to the field of regulatory peptides and neuroendocrinology. Dr. Kanoski's research—spanning the neural circuits of feeding behavior, gut-brain signaling, and metabolic dysfunction—has redefined our understanding of how peripheral peptides influence brain function and behavior. His innovative work, characterized by rigorous mechanistic studies and translational relevance, exemplifies the intellectual tradition of **Viktor Mutt**, whose pioneering discoveries laid the foundation for modern peptide research. This lectureship not only honors Dr. Kanoski's exceptional scholarship but also highlights his role as a mentor and leader shaping the future of integrative physiology.

Wednesday June 25, Afternoon

Keynote Symposium 2

Chair: **Colin Saldanha** (Director, CNB, AU)

14:00 - 14:30 **Rae Silver** (Columbia University, USA)

Circadian rhythms and the portal pathways of circumventricular organs

14:30 - 15:00 **Susan Wray (**NIH, USA)

LHRH Secretion: Why so many modulators?

15:00 - 15:30 **David Grattan** (Otago University, New Zealand)

What Prolactin teaches us about the evolution of peptide physiology

15:30 - 16:00 **Sung Han** (Salk Institute, USA)

Frequency-dependent transmitter switching in a peptidergic circuit

KS2-1 Circadian rhythms and the portal pathways of circumventricular organs

Silver, Rae
Neuroscience Department, Columbia University, New York, New York, USA

Our lab has discovered direct vascular capillary bed connections, like those of the pituitary portal system first described almost 100 years ago, between the capillary beds of each of the sensory circumventricular nuclei (CVO) and the brain. These circumventricular nuclei line the brain's ventricles. Their leaky blood vessels and large perivascular spaces represent a route whereby peptidergic output signals such as vasopressin, secreted by the brain's clock in the suprachiasmatic nucleus (SCn), can reach local targets. Timing information from the clock are then relayed and then amplified, providing a diffusible pathway to achieve global coordination of circadian clock signaling. I propose to provide a narrative that incorporates our understanding of neural and diffusible output signals of the brain's master clock in the SCN, with particular emphasis on the contribution of brain fluidic compartments and the fluids therein as a vehicle for achieving global impact on rhythms in the brain.

The author declares no competing interests.

KS2-2 GnRH secretion: Why so many modulators?

Wray, Susan
Cellular and Developmental Neurobiology Section, National insstitute of Neurological Diseases and Stroke, Bethesda, Maryland, USA

The initiation of reproductive maturation as well as maintenance of reproductive function are highly sensitive to both internal physiological factors, such as hormonal feedback, metabolic status, and stress, as well as external environmental cues, including circadian and photoperiodic changes. How these inputs are integrated in the brain, specifically, the neural circuits mediating coordinated control of puberty onset, sexual development and reproductive function are still unclear. In vertebrates, gonadotropin-releasing hormone (GnRH)-secreting neurons control fertility by secreting in the portal capillary system and thereby regulating gonadotrophs in the anterior pituitary. These neurons are localized in the forebrain, spanning from the olfactory bulbs to the level of the median eminence, with the majority of cells distributed, on either side of midline, from the rostral septal area to just caudal to the crossing of the anterior commissure. GnRH secretion in females has two distinct profiles, pulsatile and a surge. Two populations of kisspeptin neurons, via kisspeptin receptors on GnRH cells, drive the GnRH pulse (maintenance, Arc nucleus) and the surge (ovulation, AVPV nucleus). Together, these three cell populations are integral components of the hypothalamic–pituitary–gonadal (HPG) axis. In humans and mice, kisspeptin neurons are critical to normal reproduction function, with acute ablation of kisspeptin neurons in adult mice or Kisspeptin mutations/KOs inhibiting delayed/absent puberty and infertility. Similarly, infertility results if the receptor for Kisspeptin (KissR/GPR54) is mutated or KO. However, studies by Boehm's group showed that puberty onset and fertility in females was unaffected by kisspeptin

neuron ablation early in development. Consistent with this, female mice lacking neurons that express the kisspeptin receptor GPR54 were also fertile. These data suggest that there is compensation for the loss of kisspeptin neurons early in development and that initiation and completion of reproductive maturation can occur 1) in the absence of kisspeptin/GPR54 signaling and 2) with very few GnRH neurons. This raises the question, how do GnRH neurons integrate internal and external signals, under normal and abnormal conditions, to ensure the appropriate release of GnRH? Notably, GnRH neurons, in addition to expressing GPR54, express receptors for multiple neuromodulators including the anorexigenic proopiomelanocortin, as well as its product, α-melanocyte-stimulating hormone, the orexigenic neuropeptide Y, the pain modulator nociception, the mood, anxiety, satiety modulator cholecystokinin, as well as the circadian relay component vasoactive intestinal peptide, to name a few. How these circuits might act or interact over different reproductive stages will be discussed.

The author declares no competing interests.

KS2-3 What prolactin teaches us about the evolution of peptide physiology

Grattan, David R.
Centre for Neuroendocrinology, University of Otago, Dunedin, New Zealand

Prolactin is an anterior pituitary hormone well-recognized for its key role in the stimulation of milk production during lactation. Widespread distribution of the prolactin receptor in multiple tissues throughout the body, however, suggests that it also has other functions. Our focus has been on prolactin action in the brain, where the prolactin receptor is expressed in numerous populations of neurons, particularly within the hypothalamus. Prolactin is readily transported into the brain and has both acute effects on neuronal activity and transcriptional effects mediated through the JAK/STAT5 pathway. This suggests that periods of elevated prolactin secretion, such as is seen during pregnancy and lactation, will impact on function of multiple neuronal circuits. Here, I will discuss the hypothesis that the high levels of prolactin (or its placental homologue, placental lactogen) promotes adaptive changes in the maternal brain. I will present several lines of experimental evidence documenting prolactin-mediated changes in complex neuroendocrine circuits, including the induction of maternal behaviours, changes in metabolic functions, and the suppression of fertility during lactation. Collectively, this evidence that prolactin helps coordinate many of the physiological and behavioural changes that occur to help a female cope with the physiological processes of motherhood, has changed the way I think about the physiological actions of prolactin. It seems more appropriate to think of this peptide providing an afferent, interoceptive cue signalling a change in physiological state. Multiple systems that express the prolactin receptor can then adapt to this change in state. In this context, the prolactin-dependent process of lactation could be considered as but one of a repertoire of parental adaptations that is required to support parental care of developing offspring. This broader context of interpretation of peptide action is relevant to understand the multiple physiological actions of other similar systems, such as leptin and growth hormone.
The author declares no competing interests.

KS2-4 Revealing the role of peptidergic transmission in neural circuit function through presynaptic neuropeptide sensors and silencers

Han, Sung
The Salk Institute for Biological Studies

Neuropeptides play a critical role in modulating neural circuits and influencing behavior, yet their precise mechanisms of action remain largely unexplored. In this talk, I will present our latest findings on the role of neuropeptides in neural circuit function, uncovered through the use of novel genetically encoded sensors and silencers designed specifically for peptidergic transmission. By applying these innovative tools, we have identified key pathways and mechanisms by which neuropeptides regulate neuronal activity and orchestrate complex behavioral responses. This presentation will highlight the biological insights gained from these studies, offering a deeper understanding of how neuropeptide signaling shapes brain function and behavior.

Round table

Peer review & scientific publication: An open discussion

Co-Chairs: Lee Eiden and Tom Cunningham

The Round Table will be hosted by **Tom Cunningham**, UNTHSC, and **Lee Eiden**, Georgetown University.

Format: As traditional scientific publishing makes room for open publication, and new modes of peer review, the challenge of disseminating reliable scientific information, and recognizing and supporting work of impact and merit has never been more important. The panelists will take on several questions deemed by the membership of the IRPS at large to be of importance to our scientific community, and engage in informal discussion with attendees about concerns and ideas around peer review and scientific publication. It is anticipated that the Round Table will provide some perspectives on the scientific community's ownership of the final phase of the scientific process: peer-reviewed publication.

Panelists include **Rebecca L. Cunningham**, Ph.D., Dept. of Pharmaceutical Sciences, University of North Texas Health Science Center, Texas, USA, Assoc. Editor, Biology of Sex Differences; **Dave Grattan**, Ph.D., Centre for Neuroendocrinology Research, University of Otago, Dunedin, NZ, Assoc. Editor, Endocrinology, fmr Editor-in-Chief, Journal of Neuroendocrinology; **Mike Lehman**, Ph.D., Brain Health Research Institute, Kent State University, OH, USA, Editor-in-chief, Basic Strand, Journal of Neuroendocrinology; Assoc. Editor, Experimental Biology and Medicine; Subject Chair, Content Selection Advisory Board, Scopus (Elsevier). **Bob Millar**, Ph.D., Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa, fmr Editor-in-Chief Neuroendocrinology, fmr Editor-in-Chief, Clinical Strand, Journal of Neuroendocrinology and **Sarah J. Spencer**, Ph.D., School of Health and Biomedical Sciences, Royal Melbourne Institute of Technology University, Chief Editor, Neuroscience

Some questions that will be considered are: when to say yes and when to say no to peer review invitations; due diligence in peer review; what kinds of journals should early career scientists publish in; what are fair demands to make of submitting authors during review?; what are the real costs and benefits of 'open access'? what are the roles of practicing scientists in creating the future of scientific publishing?; who 'controls' the various domains of the peer review publication ecosystem?; what are the roles of society journals, OA journals and hybrid journals?

N.B. Dr. **Robert Goodman,** Deputy Editor-in-Chief of the Journal of Neuroendocrinology, will be in attendance and available to address queries during and after this panel.

Thursday June 26, Morning

Scientific Session 9

SS9: Control of peptides regulating appetitive drives and energy homeostasis

Chair: Andres Leko (Hungary)

08:00 - 08:05 Introduction

08:05 - 08:30 **Denise Belsham** (UT, Canada)

Neuropeptide regulation by hypothalamic microRNAs

08:30 - 08:55 **Andrew Lutas** (NIDDK, NIH, USA)

"cAMP-dependent mechanisms in GLP1R-expressing hindbrain neurons that cause weight loss"

08:55 - 09:20 **Andras Leko** (Semmelweis Univ., Hungary)

Sexually dimorphic effects of GHSR in diet-induced obesity

09:20 - 09:45 **John Furness** (Florey Institute, Australia)

Roles of the ghrelin receptor: investigation of its mechanism in reversing agonist action at the D2 dopamine receptor

09:45 - 09:50 General discussion/conclusion

SS9-1 **Neuropeptide regulation by hypothalamic microRNAs**

Belsham, Denise D.

Departments of Physiology and Medicine, University of Toronto, Toronto, Canada
Supported by CIHR, NSERC, CRC, BBDC and CFI.

The hypothalamus coordinates whole-body energy homeostasis and its function is dysregulated in obesity. Neuropeptide Y (NPY)/ agouti-related peptide (AgRP) neurons stimulate appetite and inhibit energy expenditure, while pro-opiomelanocortin (POMC) neurons inhibit appetite and stimulate energy expenditure. One cause of obesity is overconsumption of diets-enriched in fat and the most abundant saturated fatty acid, palmitate, disrupts hypothalamic NPY/AgRP neuron gene expression. Similarly, the endocrine disrupting chemical bisphenol A (BPA) promotes obesity and disrupts hypothalamic NPY/AgRP neuron gene expression. The mechanisms underlying the effects of palmitate and BPA are partially delineated, but the involvement of microRNAs (miRNAs), which post-transcriptionally mediate gene silencing, was not resolved. This led us to hypothesize that the obesogens, palmitate and BPA, would alter miRNA profiles from NPY/AgRP neurons and that these altered miRNAs would contribute to changes in neuropeptide levels. BPA upregulates miR-708-5p, and consistent with the effects of BPA, this miRNA induces Npy mRNA and reduces neuronatin, a proteolipid associated with weight. Palmitate upregulates miR-2137, which indirectly regulates Npy and the transcription factors, Atf3, Esr1, and Cebp-beta. Palmitate also downregulated miR-503-5p, which induces Npy mRNA levels. Finally, palmitate altered miRNAs associated with small extracellular vesicles, including miR-2137. The extracellular vesicles isolated from an untreated NPY-expressing cell line induces an increase in Pomc mRNA in a POMC-expressing cell line, but this effect was blocked after palmitate treatment of the NPY-expressing neurons. Together, these results demonstrate that miRNAs contribute to the detrimental effects of palmitate and BPA by promoting obesity by altering hypothalamic neuropeptide gene expression.

Grants from CIHR, NSERC, CRC, BBDC and CFI

SS9-2 **Neurobiology of glia-neuron interactions in the hypothalamus: Diets, feeding behavior, and neurogenesis**

García-Robles, Maria A., 1; Elizondo-Vega, Roberto, 1; Rodríguez, Andrés, 1; Durán, Carolina, 1; Recabal, Antonia, 1

Laboratorio de Biología Celular, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile

Obesity is a global health issue resulting from an imbalance between food intake and energy expenditure. The hypothalamus, the primary regulator of energy balance, integrates neuroendocrine and metabolic signals. Within the hypothalamus, the arcuate nucleus (ARC), adjacent to the median eminence (ME), plays a central role in sensing peripheral signals due to its proximity to fenestrated capillaries. Tanycytes, specialized glial cells lining the third ventricle, contact fenestrated capillaries of the ME, cerebrospinal fluid, and neuroendocrine neurons such as anorexigenic neurons, POMC. This strategic positioning lets them detect growth factors and hormonal and nutrient signals influencing food intake regulation. We

provide evidence that tanycytes regulate short- and long-term feeding behavior in high-sugar diets. In vitro and in vivo, we show that tanycytes release lactate and β-hydroxybutyrate (βHB). Long-term, we assess their proliferative and differentiative potential in response to fructose, prevalent in modern diets. Food intake, body weight, hepatic lipid accumulation, and glucose tolerance were measured, along with hypothalamic metabolomic profiles and mRNA expression in high-glucose and high-fructose-fed mice. Bromodeoxyuridine (BrdU) incorporation and hypothalamic neurosphere cultures were used to assess the proliferative capacity of neural progenitor cells (NPCs) under high-hexose conditions. Results show that lactate increases POMC neuron action potential while βHB hyperpolarizes them. In vivo, MCT1 and MCT4 inhibition in tanycytes increased food intake, and MCT2 inhibition in ARC neurons had the same effect. Enhancing tanycyte lactate production decreased food intake. Fructose significantly reduced tanycyte proliferation compared to glucose, decreasing neurosphere number and size. Newly generated neurons were also reduced in high-fructose-fed mice. Our findings suggest that tanycytes regulate feeding behavior via metabolic signaling and neurogenic potential, highlighting their role in hypothalamic energy balance regulation.

Supported by Fondecyt 1221508 and VRIM UdeC.

SS9-3 **Sexually dimorphic effects of GHSR in diet-induced obesity**

Leko, Andras 1, 2; Gregory-Flores, Adriana 1, 3; Marchette, Renata, 3

1 Clinical Psychoneuroendocrinology and Neuropsychopharmacology Section, Translational Addiction Medicine Branch, National Institute on Drug Abuse Intramural Research Program and National Institute on Alcohol Abuse and Alcoholism Division of Intramural Clinical and Biological Research, National Institutes of Health, Baltimore, Maryland, USA; 2 Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary; 3 Neurobiology of Addiction Section, National Institute on Drug Abuse Intramural Research Program, National Institutes of Health, Baltimore, Maryland, USA

Obesity is a chronic disease that leads to serious health consequences, decreased life expectancy, and significant health care costs. The stomach-derived hormone ghrelin regulates essential physiological functions. The ghrelin receptor (GHSR) has ligand-independent actions; therefore, GHSR gene deletion may be a reasonable approach to investigate the role of this system in feeding behaviors and diet-induced obesity (DIO). We investigated the effects of a long-term (12-month) high-fat (HFD) versus regular diet on obesity-related measures in global GHSR-KO and wild-type (WT) Wistar male and female rats.

The main finding is that the deletion of GHSR protects against diet-induced weight gain and decreases food intake during HFD in male but not in female rats. The reduced food intake in our model was not related to changes in locomotor activity or anxiety-like behavior evaluated in the open field and novelty-suppressed feeding tests. Using infrared thermography, we detected an increased thermogenesis in the interscapular region, where the brown adipose tissue (BAT) is mainly located, in GHSR-KO compared with WT rats in males but not females. Another novel finding of our study was that the HFD decreased brain glucose uptake in males of both genotypes, with a greater effect in WT than in GHSR-KO rats. In females, we observed the opposite, HFD attenuated glucose uptake more markedly, but also increased in certain brain areas in GHSR-KO rats. Furthermore, brain glucose uptake was increased in GHSR-KO male rats compared to WT, regardless of diet, an effect stronger in males than in females. We used RNA-sequencing to show that GHSR-KO rats have upregulated expression of genes responsible for fat oxidation in the BAT. We also tested a therapeutically promising GHSR inverse agonist for the first time in both male and female mice. PF-5190457 is the only GHSR blocker which advanced to clinical trials and is well-tolerated in humans, and in our experiments ICV administration attenuated ghrelin-induced food intake interestingly only in male, but not female mice. The effect of GHSR inverse agonism on a more hedonic aspect of food intake, the HFD-induced binge-like eating, was not sex-dependent; ICV PF-5190457 reduced HFD intake in both sexes.

In conclusion, we demonstrated a protective effect of GHSR deletion in diet-induced obesity in male, but not female, rats, which shows a dramatic sexual dimorphism. Our pharmacological studies in mice also revealed that the GHSR blockade has stronger effects in males than in females indicating that the pharmacological intervention of the GHSR in humans requires a precise assessment of the sex effects. Our results indicate an important role for GHSRs in the regulation of body weight, food intake, energy homeostasis, adipose tissue, and brain activity, and constitute a promising target for medication development for the treatment of obesity.

Supported by the NIDA IRP and the NIAAA DICBR. AL and RM were fellows from the Center on Compulsive Behaviors, NIH.

SS9-4 **Roles of the ghrelin receptor: investigation of its mechanism in reversing agonist action at the D2 dopamine receptor**

Furness, John B., 1,2; Dehkhoda, Farhad, 3; Ringuet, Mitchell T., 2; Furness, Sebastian G.B., 3,4

1 Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, Australia; 2 The Florey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia; 3 School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, Queensland, Australia; 4 Monash Institute of Pharmaceutical Sciences, Melbourne, Victoria, Australia

The ghrelin receptor (GHSR) is co-expressed with the dopamine D2 receptor (DRD2) in neurons in the hypothalamus and spinal cord. In these neurons dopamine and DRDR preferring agonists atypically cause depolarization and excitation. In other DRD2 neurons, presumed not to express GHSR, dopamine has its well described inhibitory action. Dopamine is present in descending pathways from the brain stem that impinge on the defecation centre neurons in the lumbro-sacral spinal cord, whereas ghrelin is absent from neurons innervating the defecation centre. The defecation centre neurons that express both GHSR and DRD2 receptors have been identified as parasympathetic preganglionic neurons. At these neurons dopamine elicits depolarizing excitatory responses and stimulates the parasympathetic efferent pathways to the colorectum, leading to increased colonic motility. Ghrelin receptor agonists also excite the neurons and trigger defecation. In this study we used spinal cord slices and recombinant cells to investigate the mechanisms for reversed DRD2 activity and the role for GHSR in this reversed activity. Experiments were conducted in accordance with the National Health and Medical Research Council guidelines for the care and use of animals and with approval from the University of Queensland animal use Committee and the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee (FINMH-20-024). In spinal defecation centres, those neurons excited by dopamine were also excited by GHSR agonists. In anaesthetized animals, both GHSR and DRD2 agonists, applied directly to the lumbosacral spinal cord or systemically, caused propulsive colon emptying contractions. Excitatory responses in defecation centre neurons, identified by retrograde labeling or a DRD2 reporter, were accompanied by increased iCa2+ and were prevented by depletion of intracellular calcium stores using thapsigargin. This GHSR-dependent switch in DRD2 coupling to calcium mobilization also occurred in recombinant cells but was not the result of a switch in the G protein preference of DRD2. Moreover, allostery between these receptors using ligand binding was undetectable, nor were they close enough to invoke dimerization as assessed by super-resolution microscopy (STORM imaging and quantitative deconvolution). Instead, we observe a GHSR-dependent priming of phospholipase C beta (PLC-□□), dependent on GHSR's constitutive activity. In cells co-expressing a GHSR polymorph lacking constitutive activity, DRD2 coupling to calcium was restored by priming with a concentration of ghrelin causing 10% of maximum response. This requirement for PLC-□ was seen in spinal defecation neurons, where PLC-□ inhibition reversed the dopamine response of these cells from excitatory to inhibitory. Together our data indicate that dopamine mediated excitation is dependent on GHSR constitutive activity via a dominant second-messenger switch. Neither GHSR agonism nor dimerization with D2 receptor is required. This work has broad implications for switching of metabotropic neurotransmitter responses via alternative G protein-coupled receptor modulation.

The authors declare no competing interests.

Thursday June 26, Morning

Scientific Session 10

SS10: PACAP signaling across systems: from stress circuits to metabolic control

Co-Chairs: Jessica Barson & Lee Eiden (USA)

08:00 - 08:05 Introduction

08:05 - 08:30 **Arun Anantharaman (**University of Toledo, USA)

Mechanisms for PACAP-induced depolarization leading to chromaffin cell secretion

08:30 - 08:55 **Youssef Anouar** (Univ Rouen Normandie, Inserm, France)

Central SELENOT deficiency alters GnRH levels, sexual behavior and fertility in male and female mice

08:55 - 09:20 **Jessica Barson** (Drexel University, USA)

PACAP in the paraventricular nucleus of the thalamus: Relationship with ethanol drinking and dependence.

09:20 - 09:45 **Sunny Jiang** (NIMH, USA)

Novel prefrontal cortico-hypothalamic PACAPergic projection modulates CRH neurons in PVN

09:45 - 09:50 General discussion/conclusion

SS10-1 **Mechanisms for PACAP-induced depolarization leading to chromaffin cell secretion**

Anantharam, Arun
University of Toledo, Toledo, Ohio, USA

Chromaffin cells of the adrenal medulla have an important role in the sympathetic stress response. They synthesize, store, and secrete catecholamines and other hormones into the bloodstream upon stimulation by the neurotransmitter, pituitary adenylate cyclase-activating polypeptide (PACAP). PACAP causes a long-lasting and robust secretory response from chromaffin cells. However, the identity of the cellular mechanisms underlying PACAP's actions are unclear. It was previously demonstrated that the secretory response to PACAP depends on signaling through phospholipase C ε (PLCε). The goal of this study was to elucidate the role of signaling events downstream of PLCε on the secretory response. Here it is shown that a brief exposure of chromaffin cells to PACAP causes DAG production, which is dependent on PLCε activity. PACAP stimulation also drives the translocation of protein kinase C (PKC) from the cytosol to the plasma membrane. Pharmacological inhibition of PKC with NPC 15437, a competitive inhibitor of DAG binding, significantly reduces PACAP-evoked Ca2+ signals and secretion. Moreover, NPC 15437 application abrogated PACAP-stimulated enhancements in readily-releasable pool size, Ca2+ sensitivity of granule fusion, and shift in voltage-dependence of Ca2+ channel activation. Quantitative PCR revealed PKCβ, PKCε, and PKCμ to be highly expressed in the mouse chromaffin cell. Genetic knockdown of PKCβ and PKCε inhibited the effects of PACAP, while knockdown of PKCμ had no measurable effect. This study highlights important roles for PKC signaling in a highly regulated pathway for fusion stimulated by PACAP.
The author declares no competing interests.

SS10-2 **Central SELENOT deficiency alters GnRH levels, sexual behavior and fertility in male and female mice**

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Reproductive disorders are associated with neuroendocrine dysregulation in the hypothalamic-hypophysis-gonadal (HHG) axis, which can result from a defective production and action of the neuropeptide gonadotropin-releasing hormone (GnRH), the master regulator of reproduction. We have previously shown that SELENOT, a new thioredoxin-like selenoprotein highly expressed in endocrine and neuroendocrine cells, plays a role in hormone secretion. However, whether SELENOT is involved in neuroendocrine regulatory mechanisms that impinge on vital functions such as reproduction, is totally unknown. We found that brain SELENOT deficiency results in a very strong decline in fertility and impaired sexual behavior in both male and female mice. In the brain, increased expression of GnRH was observed in the hypothalamic preoptic area and in their terminals in the median eminence of both male and female mice. This leads to a marked increase in luteinizing hormone (LH), testosterone (T) and estradiol (E2) levels, and a decrease in folliculo-stimulating hormone (FSH) level in male mice. While

female animals exhibited a severe estrous cycle disorder and a polycystic ovary syndrome (PCOS)-like phenotype, with an increase in LH and T levels and a decrease in FSH and E2 levels. SELENOT deficiency impaired LH pulse secretion in both males and females. These phenotypes are reverted after administration of a GnRH antagonist. These results demonstrate for the first time the direct role of a selenoprotein in the neuroendocrine control of reproduction and identify a new mechanism in the brain impacting GnRH neurons and regulating male and female reproductive function.
Supported by INSERM, University of Rouen Normandie, Conseil Régional de Normandie,

SS10-3 **Pituitary adenylate cyclase-activating polypeptide (PACAP) in the paraventricular nucleus of the thalamus: Relationship with ethanol drinking and dependence**

Barson, Jessica
Department of Neurobiology & Anatomy, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA

Drug addiction, according to the "dark side of addiction" hypothesis, can be conceptualized as progressing from being driven by positive reinforcement (the presentation of a desired stimulus after behavior) to being driven by negative reinforcement (the removal of an aversive stimulus after behavior). In the brain, drug dependence is thought to be associated with a persistent upregulation of stress-related neuropeptide systems, that act to drive drug intake. Thus, repeated, high levels of drug exposure and withdrawal increase levels of these neuropeptides, that then serve to promote further drug intake. Notably, one of the key roles of the neuropeptide, pituitary adenylate cyclase-activating polypeptide (PACAP), is modulating the stress response. With PACAP existing in two peptide isoforms, we have found that the less ubiquitously-expressed isoform, PACAP-27, is notably dense in cells of a specific limbic brain region, the paraventricular nucleus of the thalamus (PVT), in both male and female rats and mice. In rats engaging in binge-like ethanol drinking under the 20% ethanol intermittent access procedure, we have found that levels of PACAP mRNA and PACAP-27 peptide are increased in cells of the PVT at the end of an ethanol binge. Conversely, increased levels of PACAP-27 in the rat PVT, after injection into the PVT of a PACAP adeno-associated virus (AAV), lead to reduced binge- like ethanol drinking and preference. While these results suggest that PACAP in the PVT is upregulated by ethanol drinking and acts as an endogenous negative rather than positive feedback signal for ethanol drinking, the intermittent access procedure typically does not lead to dependence-level ethanol drinking. Therefore, to induce ethanol dependence, we gave transgenic PACAP-Cre (Adcyap1-2A-Cre) mice access to 20% ethanol with the intermittent access procedure and then exposed them to chronic intermittent ethanol (CIE) vapor. These mice after the CIE exposure demonstrated increased levels of PACAP mRNA in the PVT, not only compared to ethanol-naïve cage controls but also compared to mice that had been given access to 20% ethanol and then exposed to air (to maintain non-dependence). Notably, in ethanol drinking mice after CIE exposure, chemogenetic excitation of PVT PACAP + cells using a Cre-dependent DREADD promoted ethanol drinking in these ethanol-dependent mice. In contrast, this same excitation in air-exposed non-dependent mice instead decreased ethanol drinking, consistent with our findings with the PACAP AAV in non-dependent rats. Further, these effects of PACAP + cell excitation were not found with non-specific chemogenetic excitation of the PVT. Collectively, these results suggest that while PACAP in the PVT may serve as an endogenous negative feedback signal for non-dependent binge-like ethanol drinking, this role can switch to positive feedback after the development of ethanol dependence. With PACAP-27 being relatively selectively-expressed in the PVT, this suggests that

compounds related to PACAP-27 may be good candidates as potential therapeutics for the treatment of alcohol use disorder.
Grant: R01AA028218 F31AA031427

SS10-4 **Prefrontal cortico-hypothalamic PACAPergic projections modulate the activation of CRH neurons in the paraventricular nucleus (PVN), controlling endocrine and behavioral responses under psychogenic stress**

Jiang, Sunny Z. 1; Brancaleone, William 1; Gerfen, Charles 2; Eiden, Lee E., 1

1 Section on Molecular Neuroscience, National Institute of Mental Health Intramural Research Program, Bethesda, Maryland, USA; 2 Office of the Scientific Director, National Institute of Mental Health Intramural Research Program, Bethesda, Maryland, USA

Pituitary adenylate cyclase-activating polypeptide (PACAP) contributes to stress responses both peripherally, by acting as a secretagogue in the splanchnicoadrenomedullary system to stimulate catecholamine release during systemic and psychogenic stress, and centrally, as a neurotransmitter or neuromodulator within various brain circuits involved in psychogenic stress responses. Our recent findings suggest that PACAP-expressing neurons in the medial prefrontal cortex (mPFC) may extend projections to areas surrounding the PVN (peri-PVN regions). A large body of research has shown that corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) of the hypothalamus (CRHPVN neurons), play a critical role in activation of the hypothalamic-pituitary-adrenal (HPA) axis, orchestrating endocrine, behavioral, and autonomic responses to stress.
Depleting PACAP in mPFC neurons impairs activation of CRHPVN neurons, and reduces CRH production in response to acute restraint stress, leading to a diminished corticosterone (CORT) surge after chronic stress exposure. By employing retrograde tracing with glycoprotein-deleted, EnvA-pseudotyped rabies viral vectors (RVdGenvA) alongside a helper adeno-associated virus (AAV) expressing TVA and glycoprotein in CRHPVN neurons, we confirmed that PACAP-containing terminals from mPFC-to-PVN projections establish synaptic connections with CRHPVN neurons. Furthermore, direct infusion of PACAP1-38 into the PVN via a cannula increased c-Fos activation and elevated CRH mRNA levels in CRHPVN neurons, suggesting that PACAP released from mPFC-to-PVN terminals directly modulates neuronal activity and CRH mRNA transcription. The activation of CRHPVN neurons by PACAP from these projections during psychogenic stress influences both endocrine responses and behavioral outcomes, such as increased grooming in home cage immediately following footshock stress. PACAP deletion from mPFC PACAPergic neurons diminished this grooming response, and also attenuates c-Fos induction in CRHPVN neurons following restraint stress, while up-regulation of another IEG, Egr1 is unaffected by PACAP deletion from mPFC inputs. PACAP-dependent c-Fos activation in CRHPVN neurons after stress is blocked upon expression, in CRHPVN neurons of either the PKA inhibitor PKI, or the catalytically active phosphodiesterase protein PDE4D3. Remarkably, CRHPVN neurons maintain their primary physiological function (release of CRH leading to elevastion of CORT) in the acute psychogenic stress response even in the absence of PACAP input, even though induction of Fos—the classical immediate early gene marker of neuronal stress involvement—depends on metabotropic signaling through PACAP. These results suggest that metabotropic input to CRHPVN neurons in stress controls discrete aspects of the stress response, both immediately induced behavior and longer-term stimulus-transcription coupling to the CRH gene, while additional inputs, likely ionotropic, directly control the release of CRH at the median eminence.

Supported by the National Institute of Mental Health Intramural Research Program, Project MH002386.

Thursday June 26, Morning

Scientific Session 11

SS11: PTH2 and Catestatin: Novel peptidergic modulators of brain and behavior

Chair: Árpád Dobolyi (Hungary)

10:00 - 10:05 Introduction

10:05 - 10:30 **Gina Puska** (Univ. Veterinary Medicine, Hungary)

PTH2 (TIP39) plays a role in the control of maternal behavior

10:30 – 10:55 **Lukas Anneser** (Friedrich Miescher Inst. for Biomed. Research., Switzerland)

There are other fish in the sea: Social density encoding by the neuropeptide PTH2

10:55 - 11:20 **Árpád Dobolyi** (Eotvos Lorand Univ., Hungary)

Single nucleus sequencing of the human arcuate nucleus: insights into the role of PTH2 in prolactin secretion"

11:20 - 11:45 **Sushil Mahata** (UCSD, USA)

Chromogranin A deficiency and catestatin supplementation improve tauopathy and cognitive functions in PS19 Mice

11:45 - 11:50 General discussion/conclusion

SS11-1 **PTH2 (TIP39) plays a role in the control of maternal behavior**

Gina, Puska, 1, 2; Vivien, Szendi, 2; Máté, Egyed, 2; Melinda, Cservenák, 2; Szilvia, Bartók, 2; Usdin, Ted B., 3; Árpád, Dobolyi, 2

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The maternal brain undergoes substantial plasticity during pregnancy and the postpartum period, resulting in proper physiological and behavioral maternal phenotypes. These changes contribute to the survival of the young, which is crucial in mammals, as their offspring are not able to develop and get nurtured independently in the early postnatal period. A growing body of evidence indicates that a pivotal aspect of brain maternal adaptation following parturition is the elevated expression of the maternally induced neuropeptide, parathyroid hormone 2 (PTH2), which plays a role in lactation as well as the maintenance of maternal behavior. In rodent mothers, PTH2 expression is significantly induced in the posterior intralaminar thalamic nucleus (PIL), a brain area that has been intensely studied in recent years for its role in multisensory information processing. Intracerebrovenricular application of a PTH2 receptor antagonist reduced maternal behavior, however, the mechanisms by which PTH2 acts in the brain to promote maternal care are not yet known. The distribution of PTH2+ terminals in maternally involved brain regions suggest that these regions are potential candidates for the neuropeptide to exert its maternal actions. One such brain region is the medial preoptic area (MPOA), which is the key substrate of the maternal brain network. We have demonstrated that MPOA GABAergic neurons of female mice promote maternal care. Moreover, MPOA GABAergic neurons were found to be activated by PTH2, as evidenced by an elevation of their firing rate in a dose-dependent manner during patch clamp recordings. Additionally, MPOA receives substantial input from PIL PTH2-expressing neurons, which are supposed to transmit suckling stimuli from the pups. To examine the pup-induced activation of MPOA projecting PIL neurons, we injected a retrograde tracer into the MPOA and found that a high number of MPOA-projecting PIL neurons were activated after pup-exposure, and that the majority of these were PTH2-expressing neurons. It was established that these cells were prominently activated in mothers suckling their pups compared to mothers to whom their pups were returned but without the opportunity for suckling. Next, we examined the involvement of PIL neurons in the regulation of pup-directed behaviors. The time spent with nest building and pup-related behaviors was increased due to the chemogenetic activation of PIL neurons. In addition to the MPOA we ascertained that PIL neurons project prominently to the ventral subdivision of the lateral septum (LSv), a brain area that has long been supposed to be involved in the regulation of maternal care. Therefore, we aimed to examine the impact of PTH2+ septal PIL projection on maternal behavior. We found that GABAergic neurons activated after pup exposure in the LSv are innervated by PTH2+ terminals. Furthermore, we showed that numerous LSv GABAergic neurons express calbindin, and that mothers spend less time with licking of pups when their LSv Cb neurons are chemogenetically silenced. We also determined that maternally activated septal neurons

project predominantly to the MPOA. In conclusion, our data indicate the role of the PIL-MPOA-LSv subcircuit in the formation of the maternal phenotype based on the PTH2 neuropeptidergic system.
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SS11-2 **There are other fish in the sea: Social density encoding by the neuropeptide PTH2**

Anneser, Lukas, 1, 2; Alcantara, Ivan, 1; Gemmer, Anja, 1; Mirkes, Kristina, 1; Eilers, Tim, 1; Ryu, Soojin, 3; Schuman, Erin, 1

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While sociality is a widespread feature among animals, its genetic underpinnings are only partially understood. In an unbiased sequencing screen in zebrafish, we identified the expression of the neuropeptide parathyroid hormone 2 (pth2) to be strongly regulated by the presence of conspecifics. Pth2 transcript levels exhibit extremely rapid temporal dynamics. Within 30 minutes of exposure to conspecifics, previously isolated zebrafish showed a significant rescue of pth2 transcript levels. The link between pth2 expression and the social environment is not developmentally restricted - the acute isolation of socially reared adult fish resulted in a rapid decrease in pth2 transcript levels. Furthermore, in socially reared fish the level of pth2 expression was tightly and positively correlated with the number of fish in the environment. In a series of experiments, we identified mechanosensation via the lateral line to be the modality with which the presence of conspecifics was perceived. We furthermore analyzed the molecular composition of the pth2 expression domain, which suggested that this area is homologous to the subparaventricular region in the mammalian thalamus. Ultimately, we subjected pth2 knockout fish to a battery of behavioral tests and found mutants to display signs of increased anxiety and impaired social behavior. The data presented suggests that the neuropeptide pth2 is part of a conserved system that enables the animal to react appropriately in different social contexts.

The authors declare no competing interests.

SS11-3 **Single nucleus sequencing of the human arcuate nucleus: insights into the role of PTH2 in prolactin secretion**

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Parathyroid hormone 2 (PTH2, also known as tuberoinfundibular peptide of 39 residues) and its receptor, PTH2R, form a neuromodulatory system implicated in neuroendocrine regulation, particularly in lactation. Our studies demonstrated that PTH2 expression in the PIL is upregulated in lactating dams, and these neurons exhibit pup-induced c-Fos expression, highlighting their role in lactation. Further anatomical studies revealed that PIL PTH2 neurons receive ascending input from the spinal cord, the lateral parabrachial and gracile/cuneate nuclei, potentially relaying sensory information from the suckling stimulus. Further tracing experiments identified direct projections from PIL PTH2 neurons to the arcuate nucleus (Arc) of the hypothalamus, a critical center for prolactin regulation, where PTH2R is also highly expressed. Functional studies revealed that pharmacological blockade of PTH2R via antagonist injection into the medial hypothalamus significantly attenuated suckling-induced prolactin release, indicating that PTH2 signaling plays a key role in modulating lactational hormone secretion. Moreover, viral-mediated expression of a PTH2R antagonist in the arcuate nucleus confirmed a reduction in both basal and suckling-induced prolactin levels. Taken together, these findings establish PTH2-PTH2R signaling via PIL to arcuate projections as a fundamental mechanism in the modulation of prolactin secretion during lactation. However, it remained to be established how PTH2 can affect dopaminergic neurons in the Arc, known to control prolactin secretion, as PTH2R was not present in dopaminergic neurons. Furthermore, we investigated the presence of PTH2R in the human Arc (infundibular) nucleus for translational purposes.

The Arc is a key integrator of metabolic and neuroendocrine signals. Consequently, understanding its cellular and molecular composition is imperative for predicting neuropeptide function and intercellular communication. To this end, single-nucleus sequencing of the human Arc-median eminence region was performed, enabling high-resolution mapping of neuronal subpopulations and their signaling networks. For this purpose, Arc from 8 individual were pooled. The sequencing data were then combined with the results of the only available relevant previous human study analyzing the tuberal hypothalamic region of one individual. Our analysis identified 23 distinct neuronal subpopulations within the Arc, with neuropeptides emerging as key mediators of intercellular communication. Neuropeptide-receptor interaction modeling revealed functional compartmentalization within the Arc, with clusters expressing localized receptor distributions acting as signaling hubs. The integration of transcriptomic data with neuropeptide-receptor network analysis enabled the prediction of functional interactions governing Arc activity. The neuropeptide-receptor network map visualized the molecular framework underlying neuropeptide signaling and revealed previously uncharacterized signaling pathways. Notably, neuronal clusters expressing the PTH2R were identified, and these cells were characterized for their cell-cell communication with other Arc neurons and also the hormones acting on them. This comprehensive molecular blueprint of the human Arc demonstrates the potential of single-nucleus sequencing to predict neuropeptide function and intercellular communication, paving the way for future research into Arc-mediated regulation of metabolism and neuroendocrine function. The dataset provides a foundation for the development of targeted therapeutic strategies in metabolic and endocrine disorders.

Supported by the NAP3 project of the HAS (NAP2022-I-3/2022 and NAP2022-I-4/2022), the NKFIH OTKA grants K146077, the OTKA National Research Excellence Program 151425, and the 2024-2.1.1-EKÖP-2024-00004 University Research Scholarship Programme of the Ministry for Culture and Innovation from the source of the NKFIH Fund

SS11-4 Chromogranin A deficiency and catestatin supplementation improve tauopathy and cognitive functions in PS19 mice

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Emerging evidence links Chromogranin A (CgA), a secretory proprotein and neuroendocrine marker; with protein aggregates found in the brains of individuals suffering from neurodegenerative diseases such as Alzheimer's disease (AD). Despite growing interest in the molecular mechanisms underlying Tauopathies, including AD and corticobasal degeneration (CBD), therapeutic advances have been limited. Our study investigates the role of CgA and its derived peptide, Catestatin (CST: hCgA352–372), in Tau pathogenesis, highlighting their potential as novel translational targets for treating Tau-related neurodegenerative disorders.

We employed a Tauopathy mouse model (PS19) to study the impact of genetic deletion of CgA in the model. The resulting CgA-KO/PS19 mice exhibited significantly reduced pathological Tau aggregation and spreading, along with an extended lifespan and improved cognitive performance compared to PS19 controls. Transcriptomic and metabolomic analyses revealed dysregulation in adrenergic signaling in PS19 mice, including elevated expression of α1-adrenergic receptor (Adra1) and increased cortical epinephrine (EPI) levels - mirroring findings in AD and CBD patients. Remarkably, these abnormalities were reversed in CgA-KO/PS19 mice, implicating the CgA-EPI-Adra1 axis in the promotion of Tau pathology.

Functionally, we validated this mechanism by applying EPI or Adra1 agonists to wild-type hippocampal slice cultures, which induced Tau hyperphosphorylation and neurofibrillary tangle formation. Conversely, Adra1 antagonism suppressed these pathological changes, confirming the role of this signaling pathway in driving Tau pathology.

From a translational perspective, CST represents a highly innovative therapeutic lead. Human brain samples from patients with AD, CBD, and progressive supranuclear palsy (PSP) revealed overexpression of CgA but significantly reduced CST levels. Furthermore, lower CST levels were associated with worse cognitive outcomes based on Mini-Mental State Examination (MMSE) scores in both the cortex and hippocampus of AD patients. In the PS19 model, CST supplementation not only decreased Tau phosphorylation and aggregation but also reduced Tau seeding in ex vivo slice cultures. These effects extended to improvements in microglial activation and reductions in pro-inflammatory cytokines and chemokines - hallmarks of neuroinflammation in Tauopathies.

Crucially, we demonstrated that intraperitoneally administered CST is brain-penetrant (Cmax: 44.5 ng/g; Tmax: 0.5 hr; T1/2: 2.77 hr; Clast: 4 hr), establishing its bioavailability and supporting its development as a systemically administered therapeutic. Improvements in spatial memory and motor performance further validate CST's neuroprotective potential in vivo.

This work establishes CST as a novel peptide-based candidate for treating Tauopathies. Unlike current monoclonal antibody therapies targeting extracellular Tau aggregates, CST targets upstream regulatory pathways, offering a broader and potentially more effective disease-modifying strategy. Its endogenous origin, multimodal activity (anti-Tau, anti-inflammatory, neuroprotective), and favorable pharmacokinetics make it a uniquely positioned, first-in-class therapeutic lead. These findings lay the foundation for translational advancement of CST into Investigational New Drug (IND)-enabling studies, with the long-term goal of initiating clinical development for AD, CBD, and related disorders. As neurodegenerative diseases remain a pressing public health challenge with limited therapeutic options, CST's promise represents a significant step toward innovative, mechanism-based treatment for these devastating conditions.

Supported by grants from NIH/NIA: 1 R21 AG072487-01 and 1 R21 AG078635-01A1

Thursday June 26, Morning

Scientific Session 12

SS12: Neuropeptides in mood disorders: mechanisms linking emotion, sex, and brain state

Chair: Tom Cunningham (USA)

10:00 - 10:05 Introduction

10:05 - 10:30 **Tom Cunningham** (Texas A&M, USA)

Sex Differences in Vasopressin in an animal model of hyponatremia

10:30 - 10:55 **Mario Zetter** (UNAM/LaSalle University, Mexico)

Adult neurogenesis and migration in magnocellular AVP system

10:55 - 11:20 **Hiroe Hu (**NIH, USA)

Arginine vasopressin and mood disorders

11:20 - 11:45 **Aimin Bao** (Zhejian University, China)

The role of oxytocin in bipolar disorder: from animal model to postmortem human brain

11:45 - 11:50 General discussion/conclusion

SS12-1 **Sex differences in vasopressin release in an animal model of dilutional hyponatremia**

Cunningham, J.T., 1; Nguyen, D.H., 2; Balapattabi, K., 2; Aikins, A.O., 2

1 Texas A&M College of Medicine, Bryan, Texas, USA; 2 UNT Health Science Center at Fort Worth, Fort Worth, Texas, USA

Hyponatremia resulting from increased circulating vasopressin (AVP) increases morbidity and mortality in patients with cirrhosis and heart failure. Our laboratory has been using = an animal model of cirrhosis caused by ligation of the common bile duct (BDL) to study the central mechanisms related to pathogenesis of inappropriate AVP release. More recently, we have investigated possible sex differences in this model.

In studies of adult male and female rats, all the BDL rats had significant increases in liver to body weight ratio compared to sham rats indicating liver failure. Male BDL rats demonstrated hyponatremia along with significant increases in plasma copeptin (an AVP surrogate) and FosB expression in Supraoptic AVP neurons compared to male shams (all $p<0.05$; n=5-7). Unlike male BDL rats, the female BDL rats did not become hyponatremic and did not demonstrate Supraoptic AVP neuron activation or increased copeptin secretion as compared to sham operated females. Plasma oxytocin concentration was significantly higher in female BDL rats compared to female sham controls ($p< 0.05$; n=6-10). This increase was not observed in male BDL rats.

Ovariectomy significantly decreased plasma estradiol concentration in shams compared to intact female sham ($p< 0.05$;6-10). However, circulating estradiol concentration was significantly elevated in ovariectomized BDL (OVX BDL) rats compared to the ovariectomized sham (OVX sham) and female sham rats ($p<0.05$;6-10). To identify the source of estradiol contributing to the observed increase in OVX BDL rats, adrenal glands were collected at the end of protocol. Adrenal gland steroids were extracted to measure estradiol and its precursors, testosterone and DHEA concentration. The OVX BDL rats had significantly increased adrenal estradiol along with significant decrease in adrenal testosterone and DHEA compared to OVX sham rats (all $p< 0.05$;6-7). Female OVX BDL rats did not hyponatremia or increased copeptin secretion compared to female OVX sham rats. It is possible that the increase in adrenal estradiol compensated for the lack of ovarian estrogens in OVX BDL rats.

Spatial transcriptomics (Visium, 10x genomics) has also been used to explore possible sex-based differences in the SON that may contribute to the response to BDL. A DESeq2 analysis of SON transcripts comparing male BDL rats to controls identified 195 differentially expressed genes (DEGs) that gene ontology analysis indicated are related to membrane transport and synapse function. A similar analysis comparing the SON of BDL females to control females identified 139 DEGs related to microglia proliferation and vesicle membrane regulation. These data suggest the central plasticity contributing to the increased release of AVP and hyponatremia in male rats in response to BDL appears absent in female rats, and this may be related to the differential regulation of OXY, microglia, and vesicle regulation.

Supported by R01 HL142341 to JTC; F30 HL158145 and T32 AG020494 to DHN, and an AHA predoctoral fellowship to KB.

SS12-2 Unveiling Plasticity in the Adult Hypothalamic Vasopressin System: Neurogenesis and Migration as Mechanisms of Physiological Adaptation

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1 Department of Physiology, School of Medicine, National Autonomous University of Mexico (UNAM); 2 Department of Medicine and Health, School of Medicine, La Salle University, Mexico

The hypothalamic arginine vasopressin (AVP) system is a pivotal regulator of homeostasis and behavioral adaptation, yet its potential for adult plasticity through neurogenesis remains a frontier of discovery. While adult neurogenesis is well-established in regions like the subventricular and subgranular zones, emerging evidence suggests that the hypothalamus, particularly the AVP magnocellular neurosecretory system (AVPMNS), may also harbor neurogenic capacity. Our recent work, using multi-marker immunohistochemistry (AVP, GFAP, Ki67, DCX, BrdU), reveals low-rate neurogenesis and neuronal migration in the adult rat AVPMNS, with tangential migration routes and axonal scaffolds extending from the supraoptic and paraventricular nuclei. Osmotic stress amplifies this process, hinting at an adaptive role.
In this review, I will synthesize historical and contemporary insights into hypothalamic AVP neurogenesis, contextualizing our findings within this evolving field. I will explore how these newly generated neurons may contribute to physiological resilience, offering a perspective on the dynamic interplay between peptidergic systems and brain plasticity across the lifespan

The authors declare no competing interests. Supported by Ciencia de Frontera, CF-2023-G-243

SS12-3 Re-examining vasopressin's role in HPA-axis stress response in the context of depression and suicidality

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1 Experimental Therapeutics and Pathophysiology Branch, National Institute of Mental Health, Bethesda, Maryland, USA; 2 Department of Medicine, Georgetown University, Washington DC, USA

Arginine vasopressin (AVP) is an osmoregulatory neurohormone that also modulates social affiliative behavior and hypothalamic-pituitary-adrenal (HPA) axis stress response. Preclinical studies have shown that under chronic stress, CRH secretion is dampened from hypercortisolemia and AVP remains the main driver of ACTH release. Most studies examining the contribution of chronic stress to mood disorders and suicidality have generally focused on peripheral measurements of cortisol. This exploratory study examined plasma copeptin, a surrogate biomarker of AVP, in relation to other plasma HPA axis markers and clinical measures in patients and healthy volunteers enrolled in Evaluation of Patients with Mood and Anxiety Disorders (NCT00024635) at NIMH between 2016 to present (n=140). Basal copeptin and other HPA axis markers were compared using ANOVA in individuals with unipolar depression (n=84), bipolar depression (n=24) and healthy volunteers (n=32).

Plasma copeptin levels did not significantly differ between individuals with uni/bipolar depression and healthy volunteers ($F(2,148) = 0.149$, $p = 0.862$), nor did the copeptin-to-CRH ratio ($F(2,148) = 0.136$, $p = 0.873$). However, depressed individuals with PTSD comorbidity (n=38), compared to individuals without PTSD (n=58) and healthy volunteers (n=32), had significantly higher baseline plasma copeptin levels ($F(1,149) = 9.594$, $p = 0.002$) and copeptin-to-CRH ratio ($F(1,149) = 9.412$, $p = 0.003$). Neither CRH nor ACTH levels significantly differed by PTSD diagnosis, mood disorder, or anxiety disorder diagnoses, suggesting a possible AVP-mediated stress regulation specific to PTSD comorbid with a depressive episode. Age, sex, history of childhood trauma, chronic suicidal ideation, severity of depression, or anxiety did not significantly alter PTSD's effect on copeptin. Significant interactions were observed between PTSD comorbidity and aggression as measured with Buss-Perry Aggression Questionnaire ($F(16,57) = 2.277$, $p = 0.011$), acute suicidality in the past two weeks as measured with Columbia Suicide Severity Rating Scale ($F(4,128) = 2.729$, $p = 0.032$), and clinical severity index as measured with Clinical Global Impression Scale ($F(4,127) = 3.179$, $p = 0.016$).

For suicidal patients later enrolled in the Neurobiology of Suicide protocol (NCT02543983) and who received a single infusion of open-label intravenous ketamine at a subanesthetic dose (n=16), there was no significant changes in copeptin, CRH or copeptin-to-CRH ratio in a linear mixed model after accounting for covariates. This preliminary finding invites further exploration of neuroendocrine mechanisms of trauma-related disorders co-occurring with mood disorders, as well as other neuroendocrine treatment response biomarkers to an anti-suicidal, rapid-acting antidepressant like ketamine.

The authors extend their deepest gratitude to the clinical research participants who enrolled in our protocols and provided their samples; our team at NIMH Experimental Therapeutics and Pathophysiology branch and the NIH Clinical Center staff, including but not limited to nursing, recruiters, protocol coordinators, post-bac and post-doc research fellows, and clinicians; and the collaborators at Mayo Clinic including Dr. Anna Creo and Dr. Joshua Bornhorst for allowing access to their BRAHMS-Kryptor Copeptin assay

SS12-4 The role of oxytocin in bipolar disorder: from animal model to postmortem human brain

Bao, Aimin
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Bipolar disorder (BD) and major depressive disorder (MDD) are the two main types of mood disorders, which share common characteristics in stress-related brain circuits, yet they also exhibit distinct symptomatology. We aim to search for biomarkers that distinguish these two mood disorders. Increased plasma oxytocin (OT) levels are a promising biomarker for BD. We have earlier stimulated OT neurons in the mice hypothalamic paraventricular nucleus (OT PVN) and triggered mood changes. However, the contribution of the supraoptic nucleus (SON) OT neurons (OT SON) to BD remains unknown, although the SON is a major part of the central OT system. Here, we present four independent experiments for acute or chronic chemogenetic activation of OT PVN or OT PVN+SON neurons in OT-cre mice. In addition, following chronic activation we measured the mRNA expression of stress-related molecules in the medial prefrontal cortex (mPFC). We observed that acute activation of OT PVN+SON led to slight

mania-like behaviors which may be regarded as anti-depression/anti-anxiety effects. Chronic activation of OT PVN led to anxiety/depression-like behaviors in male mice, while chronic activation of OT PVN+SON led to mania-like behaviors both in male and female mice. In addition, the alterations in stress-related molecules in the mPFC showed clear sex differences. Subsequently we studied in postmortem human brain material to validate our results in animal models. Alterations in levels of OT-immunoreactivity (ir) and for the first time in vasopressin (AVP)-ir were determined in the SON and PVN among patients with BD, MDD, and matched controls. We observed a significantly increased OT PVN -ir but relatively stable AVP PVN -ir in male BD, and a significantly decreased AVP PVN -ir but relatively stable OT PVN -ir in female BD patients. A significantly increased ratio of OT-ir/AVP-ir was observed only in BD patients in both, the PVN and SON. No significant changes in OT-ir or AVP-ir were found in MDD patients compared with controls. Our data show a clear disease- and sex-specificity of the OT and AVP changes in BD. The ratio between OXT/AVP seems to be the biomarker to distinguish between BD and MDD. This possibility will now be tested in mood disorder patients.
The authors declare no competing interests.

SS12-5 **Hypocretin-1/hypocretin receptor 1 regulates neuroplasticity and cognitive function through hippocampal lactate homeostasis in depressed model**

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Cognitive dysfunction is not only a common symptom of major depressive disorder, but also a more common residual symptom after antidepressant treatmentand a risk factor for chronic and recurrent disease. The disruption of hypocretin regulation is known to be associated with depression, however, their exact correlation is remains to be elucidated. Hypocretin-1 levels were increased in the plasma and hypothalamus from chronic unpredictable mild stress (CUMS) modelmice. Excessive hypocretin-1 conducted with hypocretin receptor 1 (HCRTR1) reduced lactate production and brain-derived neurotrophic factor (BDNF) expression byhypoxia-inducible factor-1α (HIF-1α), thus impairingadult hippocampal neuroplasticity, and cognitive impairment in CUMS model. Subsequently, we found that HCRTR1 antagonists could reverse these changes. The direct effect of hypocretin-1 on hippocampal lactateproduction and cognitive behavior was further confirmed by intraventricular injection of hypocretin-1 and microPET-CT in rats. In addition, we further validated these mechanisms in astrocytes and neurons in vitro.Moreover, these phenotypes and changes in molecules of lactate transport pathway could be duplicated byspecifically knockdown of HCRTR1 in hippocampalastrocytes. In summary, the results provide molecular and functional insights for involvement of hypocretin-1-HCRTR1 in altered cognitive function in depression.
The author declares no competing interests.

Thursday June 26, Afternoon

PL6: Plenary Lecture. A role for GnRH in the control of cognition…and more?

13:00 - 13:45 **Vincent Prévot** (Lille, France)

introduced by **Ruud Buijs (Mexico)**

Prévot, Vincent

Laboratory of Development and Plasticity of the Neuroendocrine Brain, Lille Neuroscience & Cognition, UMR_S1172, Lille, France

Pulsatile secretion of gonadotropin-releasing hormone (GnRH) is essential for activating and maintaining the function of the hypothalamic-pituitary-gonadal (HPG) axis, which controls the onset of puberty and fertility. Two provocative recent studies suggest that, in addition to controlling reproduction, the neurons in the brain that produce GnRH are also involved in the control of postnatal brain maturation, odor discrimination, and adult cognition. I will discuss the development and establishment of the GnRH system, and especially the importance of its first postnatal activation, a phenomenon known as minipuberty, to its later functions, reproductive and non-reproductive. In addition, I will discuss the beneficial effects of restoring physiological, i.e. pulsatile, GnRH levels on olfactory and cognitive alterations in Down syndrome and preclinical models of Alzheimer's disease, as well as the risks associated with long-term continuous, i.e. non-physiological, GnRH administration in certain disorders. Finally, I will discuss the intriguing possibility that pulsatile GnRH therapy may hold therapeutic potential for the management of some neurodevelopmental cognitive disorders as well as pathological aging in the elderly.

Supported by European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement n◦ 725149

About the speaker

Vincent Prevot is Senior Research Director at Inserm and heads the Development & Plasticity of the Neuroendocrine Brain laboratory within the Lille Neuroscience & Cognition Center, University of Lille. As President of the International Federation of Neuroendocrinology from 2020 to 2024, he championed integrative approaches linking metabolism, reproduction, and brain health. Prevot's work revealed how tanycytes and other glial cells gate peripheral metabolic cues and remodel GnRH networks, redefining how the hypothalamus synchronizes fertility with energy status. Most recently his group showed that GnRH itself modulates sensory processing and higher cognitive circuits; pulsatile GnRH therapy restored memory and attention in mouse models and in a pilot study of adults with Down syndrome, opening translational avenues for neurodegenerative disorders. His RegPep25 plenary lecture, **"A Role for GnRH in the Control of Cognition… and More,"** will spotlight these discoveries and their implications for healthy brain ageing.

Friday June 27, Morning

SL: Special Lecture 1. Descending peptidergic control of chronic mechanical pain

08:00 - 08:45 **Xiaoke Chen** (Stanford USA)

introduced by **Lee Eiden** (USA)

Abstract

Descending peptidergic control of chronic mechanical pain

Wang, Qian, 1; Shim, Hyingeun, 1; Lee, Hankyu, 2; Yuan, Yuan, 1; Qi, Wei, 1; Nachtrab, Gregory, 1; Lee, Joo-Han, 1; Lu, Jacqueline, 1; Duan, Bo, 2*, Chen, Xiao-ke, 1*

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Persistent mechanical pain caused by inflammation or nerve injury poses a significant clinical challenge, often defying effective treatment. The GABAergic □-opioid receptor expressing spinal cord-projecting neurons in the rostral ventromedial medulla (OPRM+ RVMSC neurons) is essential for driving chronic mechanical pain by activating the ascending spinothalamic tract, though the underlying molecular and circuitry mechanisms remain unclear.

Combining molecular profiling, chemogenetic manipulation, electrophysiology and behavioral assays, we identified a neuropeptide, rather than the conventional neurotransmitter GABA, from OPRM+ RVMSC neurons mediates injury-induced neuropathic pain. We further revealed a spinal circuit that gates the transmission of non-painful touch information into a pain sensitization circuit.

Our findings identified novel molecular and circuit targets for treating injury or inflammation caused pain sensitization.

Supported by NIH R01NS129834 to XC and NIH R01NS109170 to BD

About the speaker

Xiaoke Chen is Associate Professor of Biology at Stanford University and a member of the Wu Tsai Neurosciences Institute, where his laboratory dissects circuit and molecular mechanisms that convert acute nociception into persistent pain. Combining viral tracing, optogenetics and single-cell transcriptomics, Chen recently uncovered a collicular–rostral ventromedial medulla pathway in which the neuropeptide dynorphin and the pseudokinase CaMKv cooperate to drive chronic mechanical hypersensitivity—findings now fueling non-opioid gene-therapy strategies His broader programme explores how descending peptidergic signals intersect with monoaminergic networks to link pain, affect and addiction. Trained at the Institute of Neuroscience, Chinese Academy of Sciences (Ph.D.) and Columbia University (post-doc), he joined Stanford in 2013 and has since been recognised with the Firmenich Next Generation Chair in Neuroscience, Sloan Research Fellowship and McKnight Scholar Award At RegPep25 he will deliver the plenary lecture “Descending Peptidergic Control of Chronic Mechanical Pain,” outlining therapeutic avenues that emerge from decoding long-range peptide signalling.

Friday June 27, Morning

SL: Special Lecture 2. Prefrontal cortical neuropeptidergic control of threat processing and circuit function

10:00 - 10:45 **Hugo Tejeda** (NIMH, NIH, USA)

introduced by **Susan Wray** (USA)

Prefrontal cortical neuropeptidergic control of threat processing and circuit function

Tejeda, Hugo
Unit on Neuromodulation and Synaptic Integration, National Intitute of Mental Health Intramural Research Program, Bethesda, Maryland, USA

Combining molecular profiling, chemogenetic manipulation, electrophysiology and behavioral assays, we identified a neuropeptide, rather than the conventional neurotransmitter GABA, from OPRM+ RVMSC neurons mediates injury-induced neuropathic pain. We further revealed a spinal circuit that gates the transmission of non-painful touch information into a pain sensitization circuit.

Supported by a Brain and Behavior Research Foundation Young Investigator Award (HAT), the NIH BRAIN Initiative (1UF-1NS133763-01), and the NIMH Intramural Research Program.

About the speaker

Hugo A. Tejeda is a Stadtman Investigator and Chief of the Unit on Neuromodulation and Synaptic Integration at the National Institute of Mental Health. After earning his Ph.D. at the University of Maryland School of Medicine and completing post-doctoral training at NIDA, he launched his independent laboratory at NIMH in 2018 to probe how neuromodulatory peptides tune limbic circuitry under normal and pathological conditions. Using viral tracing, in vitro and in vivo electrophysiology, and fiber photometry, Tejeda has shown that dynorphin–κ-opioid signaling selectively gates glutamatergic and GABAergic inputs to the medial prefrontal cortex and nucleus accumbens, thereby rebalancing excitation–inhibition ratios that shape affect, motivation, and cognitive control
nature.com. His integrative framework links peptide dysregulation to depression, addiction, and stress disorders and suggests novel circuit-targeted therapies. In recognition of these contributions he received a Presidential Early Career Award for Scientists and Engineers and was named an NIH Distinguished Scholar. At RegPep25 he will present new work on peptide-based circuit dysfunction in psychiatric disease and the therapeutic avenues it opens.

Friday June 27, Morning

Closing Plenary Lecture (PL7):

Dead brains tell lively stories

11:00 - 11:45 Dick Swaab

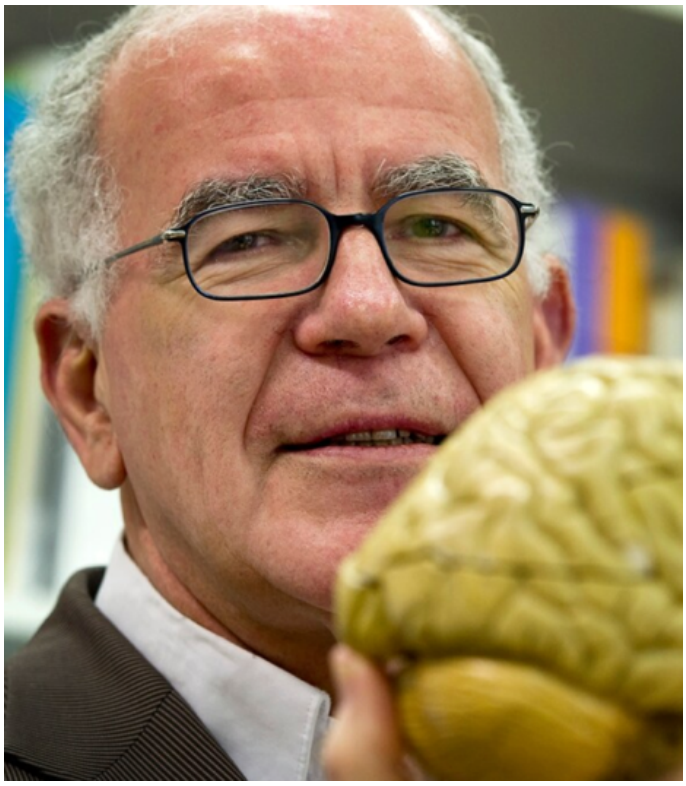
Netherlands Institute for Neuroscience, Amsterdam Department

Neuropsychiatric Disorders

Introduced by **Ruud Buijs** (Mexico)

Postmortem human brain studies can be used i) to validate animal experimental data, ii) to study conditions for which animal models are not available like gender-identity disorders or suicide or iii) to search for novel therapeutic targets for neuropsychiatric disorders. Careful matching for putative confounding factors is essential. In transsexual people we observed a reversal of the sex difference in the central nucleus of the Bed Nucleus of the Stria terminalis (BSTc) that was visualized by somatostatin or VIP staining. The size agreed with their gender identity and not with their genetic sex. Data of persons with abnormal adult sex hormone levels showed that the size is determined in early development, as it is in rodents. This has led to the acceptance of a law in the UK that made it possible to change the sex in birth certificate and passport. Our publications have also been used in the European Court for the same purpose. Recent epidemiological studies have associated peripheral progesterone levels with suicide risks in humans. We found in the infundibular nucleus of the hypothalamus an elevation of the number of pro-opiomelanocortin+ (POMC) neurons that co-expressed the progesterone receptor, a phenomenon that was related to suicide in patients with mood disorders. Mood disorder donors who died of legal euthanasia had a higher proportion of progesterone receptor co-expressing POMC+ neurons than mood disorder patients who died naturally. This indicates that a strong death wish may be progesterone-associated. Our findings may have implications for users of progestagen containing contraceptives who have mood disorders and suicidal tendencies. We found in post-mortem material that diminished vasopressin mRNA in the biological clock was the earliest biomarker for Alzheimer, occurring in Braak stage 1 and 2 well before other symptoms. Subsequently, in a well-controlled clinical trial in nursing homes, increased light (1000 lux) during the day together with 2.5 mg melatonin 2 hours before sleeping appeared to diminish nightly restlessness, improved circadian rhythms, mood and cognition in late state dementia patients. We observed a loss of oxytocin in the Paraventricular Nucleus of Prader-Willi patients. Oxytocin is inhibiting eating behavior and acts as a social peptide. Trial with oxytocin are now performed in Prader-Willi patients and other eating disorders to inhibit eating behavior, in Autism spectrum disorder and schizophrenia to improve social cognition and theory of mind and in schizophrenia to reduce symptom severity. For patients with narcolepsy, who have a strong loss of orexin/hypocretin neurons, orexin agonists are a promising treatment. The menopausal drop in estrogens cause a strong activation of hypothalamic Neurokinin-B neurons in women that is responsible for hot flushes and night sweats. Neurokinin-B antagonists are a promising, non-hormonal treatment. Concluding, hypothalamic research is now in the stage of clinical applications and post-mortem human tissue, as we obtain now for 40 years by the Netherlands Brain Bank, is a basis for this progress.

About the speaker



Dick F. Swaab, M.D., Ph.D. is Group Leader of the Neuropsychiatric Disorders team at the Netherlands Institute for Neuroscience and Professor of Neurobiology at the University of Amsterdam. In 1985 he founded the Netherlands Brain Bank, creating an unparalleled post-mortem resource that has supplied more than 5,000 well-documented brains to investigators worldwide and transformed research on neurodegeneration, psychiatric illness, and development.

Over five decades of "living with dead brains," Swaab's own work has redrawn the atlas of the human hypothalamus. He identified sexually dimorphic nuclei and showed that vasopressin- and oxytocin-expressing neurons differentiate under perinatal hormonal control, providing the first anatomical evidence that gender identity and sexual orientation are rooted in brain structure rather than choice. Subsequent studies linked hypothalamic peptide imbalance to Alzheimer's disease, major depression, and circadian disruption, underscoring peptide systems as lifelong determinants of brain health.

Swaab has authored more than 600 peer-reviewed papers (h-index ≈ 135) and mentored over 80 Ph.D. graduates. His popular-science book ***We Are Our Brains*** (Dutch original 2010; English translation 2014) spent more than two years on the Dutch bestseller list—seven weeks at #1—selling over 450,000 copies and appearing in 18 languages; it offers an accessible "neurobiography" that follows the brain from conception through ageing, illustrating how neural chemistry shapes everything from sexuality to spirituality. A children's version, *You Are Your Brains*, extends his outreach to younger audiences.

Recognized as a Knight of the Order of the Netherlands Lion and recipient of the Alzheimer's Association Lifetime Achievement Award, Swaab continues to influence public discourse through best-selling books and media appearances.

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